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*INDEX NUMBER*

VOL. 34 DECEMBER, 1949 No. 12

# The Journal of Laboratory and Clinical Medicine

*The Official Publication of the Central Society for Clinical Research*

## Clinical and Experimental

Amino Acid Excretion in Degenerative Diseases of the Nervous System. Huntington Porter, M.D., Boston, Mass. ---- 1623

Parenteral Nutrition. X. Observations on the Use of a Fat Emulsion for Intravenous Nutrition in Man. Sherwood W. Gorens, M.D., Robert P. Geyer, Ph.D., LeRoy W. Matthews, B.S., and Fredrick J. Stare, M.D., Boston, Mass. 1627

The Absorption and Disposition of Orally Administered $I^{131}$-Labeled Neutral Fat in Man. Malcolm M. Stanley, M.D., and Siegfried J. Thannhauser, M.D., Ph.D., Boston, Mass. ---- 1634

The Effect of Splenectomy on the Toxicity of $SR^{89}$ to the Hematopoietic System of Mice. Leon O. Jacobson, M.D., Eric L. Simmons, Ph.D., and Matthew H. Block, M.D., Ph.D., Chicago, Ill. ---- 1640

Japanese B Encephalitis: Report of Five Cases. Lieutenant (j.g.) N. F. Wyatt, Medical Corps, United States Navy ---- 1656

Pancreatitis in Infectious Mononucleosis. James Myhre, M.D., and Samuel Nesbitt, M.D., Ph.D., Minneapolis, Minn. ---- 1671

A Case of Congenital Idiopathic Methemoglobinemia. Ben Fisher, M.D., and J. Waide Price, Ph.D., Cleveland, Ohio ---- 1676

## Laboratory Methods

A Simplified Turbidimetric Method of Aureomycin Assay for Capillary Blood and Other Body Fluids. Coleman M. Whitlock, Jr., M.D., Andrew D. Hunt, Jr., M.D., and Sylvia G. Tashman, A.B., Philadelphia, Pa. ---- 1682

A Micromethod for Blood Penicillin Assay. Gavin Hildick-Smith, M.D., and Mary Fell, B.S., Philadelphia, Pa. ---- 1687

The Production of Antirabbit Hemolysin. Ardzroony A. Packchanian, Ph.D., Galveston, Texas ---- 1692

Differentiation and Enumeration of Eosinophils in the Counting Chamber With a Glycol Stain; A Valuable Technique in Appraising ACTH Dosage. Theron G. Randolph, M.D., Chicago, Ill. ---- 1696

*(Contents continued on inside front cover)*

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## *Contents—(Continued from front cover)*


Warburg Manometer Calibrator. Arnold Lazarow, M.D., Ph.D., Cleveland, Ohio 1702

A Newly Developed Electromagnetic Flow Meter. A. W. Richardson, Ph.D., J. E. Randall, B.S., and H. M. Hines, Ph.D., Iowa City, Iowa ---------- 1706

Evaluation of a New Capillary Resistometer: The Petechiometer, Edward E. Brown, M.D., Ashland, Ore. ---------- 1714

A Simplified Venous Occlusion Method of Digit Blood Flow Estimation Using the Burch-Winsor Plethysmograph. Charles W. Robertson, M.D., Douglas A. Farmer, M.D., and Reginald H. Smithwick, M.D., Boston, Mass. ---------- 1718


### Proceedings of the Twenty-Second Annual Meeting of the Central Society for Clinical Research


Abstracts—Concluded:

Experimental Production of Megaloblastic Anemia; An Interrelationship Between Ascorbic Acid and Pteroylglutamic Acid. Charles D. May, M.D., E. N. Nelson, M.D. (By Invitation), and R. J. Salmon, M.Sc. (By Invitation)----- 1724

Certain Effects of Chemotherapy on the Fecal Aerobic and Anaerobic Bacteria of Patients With Chronic Ulcerative Colitis. Homer C. Marshall, M.D. (By Invitation), Walter L. Palmer, M.D., and Joseph B. Kirsner, M.D. ---------- 172'

Alterations in Testicular Structure and Function in Organic Disease of the Pituitary. E. Perry McCullagh, M.D., Allen Gold (By Invitation), and J. B. Ralph McKendry (By Invitation) ---------- 1

Neutropenia and Splenomegaly Associated With Rheumatoid Arthritis. Max M. Montgomery, M.D., and Robert L. Grissom, M.D. ----------

Quantitative Response of the Prostatic Acid Phosphatase of the Immature Rat to Chorionic Gonadotrophin and Extracts of Male Urine. E. Perry McCullagh, M.D., and C. A. Schaffenburg, M.D. (By Invitation) ----------

A Comparison of Different Regimens in the Treatment of Hepatic Cirrhosis. Gordon R. Morey, M.D. (By Invitation), and Robert M. Kark, D.C.H.----



*(Contents continued on advertising page 36)*



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Vol. 34, No. 12, December, 1949. The Journal of Laboratory and Clinical Medicine is published monthly by The C. V. Mosby Company, 3207 Washington Blvd., St. Louis (3), Mo. Subscription Price: United States, its possessions, Pan-American Countries, $8.50 per year; Canada, $9.50; Foreign, $9.50. Subscriptions to medical students, interns and residents, half price. Entered as Second-Class Matter, October 8, 1915, at the Post Office at St. Louis, Missouri, under Act of March 3, 1879. Printed in the U. S. A.

# AMINO ACID EXCRETION IN DEGENERATIVE DISEASES OF THE NERVOUS SYSTEM

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THE recent investigations of Uzman and Denny-Brown[1] and Cooper and co-workers[2] have shown excessive urinary amino acid excretion to be a consistent feature of hepatolenticular degeneration even in early and mild states of the disease. Occurrence of this amino-aciduria with only minimal evidence of general liver dysfunction suggests that it may represent the result of a specific metabolic defect and that such defect may have a direct relationship to cerebral degeneration. It therefore seemed of interest to study amino acid excretion in a group of patients with other syndromes characterized by chronic neuronal degeneration, particularly those of familial type.

A second type of metabolic abnormality in Wilson's disease is indicated by the marked increase in copper content of both brain and liver noted by Haurowitz,[3] Glazebrook,[4] and in the well-controlled observations of Cumings.[5] Mandelbrote and associates[6] reported high urinary copper output in a single case of Wilson's disease and found that in this condition, as in normal persons, the administration of BAL (2, 3-dimercaptopropanol) greatly increased copper excretion. BAL might thus provide a tool for the demonstration of a possible relation between these two established biochemical abnormalities. The effect on the amino-aciduria of a marked, though transient, disturbance of copper metabolism as produced by BAL was therefore investigated.

## MATERIAL AND METHODS

Amino acid excretion was studied in representative cases of Huntington's chorea, paralysis agitans, dystonia musculorum deformans, familial spastic paraplegia associated with mental defect, and hepatolenticular degeneration. Urine samples were collected over a known time interval, usually between two and three hours. In all female subjects, and in male subjects uncooperative because of intellectual impairment, collection was by in-lying catheter. The total fasting urinary $\alpha$-amino acid nitrogen excretion in milligrams per hour was determined by the ninhydrin-carbon dioxide method of Van Slyke, MacFadyen, and Hamilton.[7] Analyses for tryptophane were carried out by the *p*-dimethylaminobenzaldehyde method of Bates,[8] after mercuric sulfate precipitation as described by Folin and Ciocalteu.[9] Xanthurenic acid was tested for by the color reaction with ferric chloride reported by Lepkovsky and co-workers.[10] Chromatographic analyses for the presence of unusually large amounts of individual amino acids were performed by the method of Dent[11] using the one-dimensional technique with phenol as the solvent. In patients with hepatolenticular

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This work was done with the assistance of a grant from the Harrington Fund and completed during the tenure of a Public Health Service Postdoctorate Research Fellowship of the National Institutes of Health.

Received for publication, Aug. 8, 1949.

degeneration, copper was estimated by the diethyldithiocarbamate method of Eden and Green.[12] Acid-cleaned glass and water redistilled over glass were used throughout in the collections and analyses for copper. Blank determinations were performed to correct for minute amounts of copper in the reagents.

## RESULTS

With the exception of hepatolenticular degeneration, none of the conditions studied showed a significant elevation in urinary total α-amino acid nitrogen excretion per hour when compared with control values on eight individuals which averaged 4.7 mg. per hour, range 3.5 to 6.8 mg. per hour (Table I). Excretion of tryptophane and xanthurenic acid did not differ grossly from normal with the relatively crude methods employed. Chromatographic studies gave no indication of excessive excretion of any one amino acid. The usual finding by this method was two distinct spots with $R_f$* values of about 0.34 and 0.51, identified by superposition with knowns as glycine and alanine. As in normal urine, a third faint spot with an $R_f$ value of about 0.83 also was present occasionally.

TABLE I. TOTAL URINARY α-AMINO NITROGEN IN DEGENERATIVE DISEASES OF THE CENTRAL NERVOUS SYSTEM

| PATIENT | α-N (MG. PER HR.) | PATIENT | α-N (MG. PER HR.) |
|---|---|---|---|
| Huntington's chorea | | Dystonia musculorum deformans | |
| M. H. | 4.4 | E. S. | 3.2 |
| | 4.0 | | 2.3 |
| | | | 7.9 |
| R. G. | 3.8 | | |
| | | W. McC. | 3.6 |
| C. W. | 5.2 | | |
| | 4.4 | | |
| | | Familial spastic paraplegia | |
| T. B. | 3.5 | W. McD. | 3.3 |
| | 3.7 | | 3.6 |
| | | | |
| Paralysis agitans | | J. J. | 4.1 |
| A. H. | 4.9 | | 4.0 |
| | 4.1 | | |
| | | Hepatolenticular degeneration | |
| L. F. | 3.0 | A. G. | 19.4 |
| | 3.6 | | 16.5 |
| | | | |
| | | E. R. | 11.0 |
| | | | |
| | | M. R. | 12.0 |
| | | | 13.7 |

The average value of 14.5 mg. α-amino nitrogen per hour in the three cases of hepatolenticular degeneration confirms the marked increase in urinary total α-amino nitrogen excretion previously observed in this disease by Uzman and Denny-Brown. The absence of such a finding in the other conditions studied suggests that α-amino nitrogen determination may be of value in the differential diagnosis between Wilson's disease and other diseases which occasionally give similar clinical pictures.

The initial urinary copper values of 23.5 and 11.0 γ per hour indicate a definitely increased copper excretion in hepatolenticular degeneration when

*$R_f$ indicates the ratio of the distance along the filter paper moved by the amino acid to the total distance traveled by the solvent.

compared with control figures on four individuals averaging 5.3 γ per hour, range 3.0 to 7.9 γ per hour. These controls are in good agreement with the more extensive observations of Mandelbrote and co-workers[6] who reported a normal average of 4.85 γ per hour, range 2.1 to 9.0 γ per hour.

Administration of BAL sufficient to increase the copper excretion by more than seven times the pre-BAL figure did not reduce the amino-aciduria of hepatolenticular degeneration to the control range (Table II). In the first patient injection of BAL was followed by a reaction characterized by profuse sweating, congestion of the sclera, puffiness of the face, nausea, vomiting of about 100 c.c. of bright red blood, and almost complete anuria which persisted for about one-half hour. Intravenous saline infusion was started as soon as the severity of the reaction became evident. It is possible that the apparent drop in amino acid excretion in the first period immediately after BAL may be in part accounted for by this transient extreme oliguria. Subjective improvement, diminished tremor, and increased activity about the ward were noted in this patient following BAL, but it is possible that these changes did not exceed the range of natural fluctuation in the disease. This improvement has persisted during the five months of observation since BAL administration. In the second patient the dose of BAL was smaller, reaction was minimal, and urine volume was maintained by constant intravenous saline infusion throughout the experiment. Amino acid output under these conditions showed no significant change. Subjectively and objectively the clinical status of this patient remained unaltered.

TABLE II. THE EFFECT OF BAL ON AMINO-ACIDURA IN HEPATOLENTICULAR DEGENERATION

| TIME OF COLLECTION PERIOD (MIN.) | URINE VOLUME (ML. PER HR.) | α-AMINO N (MG. PER HR.) | URINE COPPER (γ PER HR.) |
|---|---|---|---|
| Patient A. G.: Received BAL 7 mg. per Kg. intramuscularly | | | |
| 90 min. before BAL | 213 | 16.5 | 23 |
| BAL administered | | | |
| 0-125 min. after BAL | 143 | 10.6 | 181 |
| 125-190 | 495 | 20.8 | 133 |
| 190-357 | 439 | 19.0 | 61 |
| 357-482 | 322 | 17.9 | 40 |
| Patient E. R.: Received BAL 4 mg. per Kg. intramuscularly | | | |
| 125 min. before BAL | 85 | 11.0 | 11 |
| BAL administered | | | |
| 0-69 min. after BAL | 76.5 | 11.7 | 101 |
| 69-131 | 136 | 14.5 | 87 |
| 131-256 | 190 | 12.3 | 26 |

It is necessary to emphasize that the small number of observations must limit conclusions from the data here reported. Further, chromatography and the methods used for estimating tryptophane and xanthurenic acid were employed only as screening procedures to detect changes of marked degree. However, they did serve to diminish the likelihood that alteration in excretion of individual amino acids was occuring without change in the total α-amino nitrogen. The method used for determination of total α-amino nitrogen, on the other hand, is one of great precision which appears to exclude increase in total amino acid excretion in Huntington's chorea, paralysis agitans, dystonia musculorum deformans, and familial spastic paraplegia and to differentiate these

syndromes from Wilson's disease. Increased copper excretion was observed in two cases of hepatolenticular degeneration, but no significant change in total amino acid excretion was found at the time of a marked rise in copper output.

## SUMMARY

1. No evidence of abnormal amino acid excretion was found in cases of Huntington's chorea, paralysis agitans, dystonia musculorum deformans, or familial spastic paraplegia. These negative results are contrasted with the consistent amino-aciduria of hepatolenticular degeneration.

2. Increased urinary copper excretion was observed in two cases of hepatolenticular degeneration.

3. Administration of BAL sufficient to increase the copper output by sevenfold did not significantly reduce the amino-aciduria of hepatolenticular degeneration.

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# PARENTERAL NUTRITION

## X. Observations on the Use of a Fat Emulsion for Intravenous Nutrition in Man

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AN EMULSION of fat consisting of 15 per cent coconut oil, 4.3 per cent dextrose, and a combination of 0.5 per cent soybean phosphatides and 1 per cent polyglycerol esters as stabilizers was reported by this laboratory to have been administered successfully to animals (rats, cats, rabbits, and dogs) and to man.[1] The size of the fat particles of this emulsion was below 1 $\mu$ in diameter for the most part and none were larger than 3 microns. The preparation of this emulsion has been fully described in earlier papers.[2, 3, 4] While this emulsion (referred to as Emulsion 41 in the earlier report[1]) has repeatedly been used with success in man, it was pointed out[1] that it was not completely satisfactory from the clinical viewpoint because after a period of approximately one month it developed pyrogenic properties. The purpose of this paper is to present additional information on the use of Emulsion 41 in man, although neither the cause nor prevention of the slow development of pyrogenic activity is yet clearly understood. However, these studies were all carried out with batches of emulsion less than four weeks of age and, hence, within the time period that it may be used clinically without pyrogenic reaction.

### EXPERIMENTAL

The emulsion used in the present study was of the composition of Emulsion 41[1] and was prepared in this laboratory. Before clinical use, the sterility of each batch of emulsion was determined and the following routine tests were done: (a) rapid injection (15 ml. per kilogram body weight) intravenously into three adult rats daily for seven days and subsequent post-mortem examination, (b) rapid injection (15 to 20 ml. per minute) intravenously into one adult dog for two successive days in an amount of 5 Gm. of fat per kilogram of body weight, and (c) rapid injection (10 ml. per kilogram body weight) intravenously into adult rabbits for pyrogenic assay. Every batch of emulsion had to pass satisfactorily all of these tests before it was used in the clinic. On the average, an emulsion had been prepared for approrimately a week before it was used in man. Plastic disposable infusion sets were employed, and an infusion rate of 4 to 5 ml. per minute was used in adults and 1 to 3 ml. per minute in children, depending on the weight of the child. Viscosity of the emulsions was such that a No. 26 needle could be employed when necessary.

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From the Department of Nutrition, Harvard School of Public Health, the Department of Biological Chemistry, Harvard Medical School, and the Medical Clinic, Peter Bent Brigham Hospital.

Supported in part by grants-in-aid from the National Dairy Council, Chicago, Ill., the Upjohn Company, Kalamazoo, Mich., the Nutrition Foundation, Inc., New York, N. Y., the Milbank Memorial Fund, New York, N. Y., and the Cancer Research Grants Branch, National Cancer Institute, Bethesda, Md.

Received for publication, Aug. 10, 1949.

TABLE I. SUMMARY OF INTRAVENOUS ADMINISTRATION OF A 15 PER CENT FAT EMULSION (EMULSION 41) TO A VARIETY OF PATIENTS

(EACH 100 ML. OF EMULSION PROVIDES APPROXIMATELY 160 CALORIES)

| PATIENT AND DIAGNOSIS | DAILY QUANTITY OF FAT GIVEN I.V. | RATE OF INFUSION (ML./MIN.) | DAYS INFUSION GIVEN | REACTION OF PATIENT |
|---|---|---|---|---|
| Pt. 1 (4B708), 37 kg., 54 yr., W., F.: Anorexia and weight loss due to intractable epigastric pain | 500 ml. | 4-5 | 10 | None |
| Pt. 2 (4B529), 40 kg., 24 yr., W., M.: Ileostomy for ulcerative colitis | 600-900 ml. | 4-5 | 10 | None |
| Pt. 3 (H9129), 60 kg., 73 yr., W., M.: Carcinoma of the rectum with metastases | 300-400 ml. | 4-5 | 10 | None |
| Pt. 4 (H8718), 40 kg., 55 yr., W., F.: Rheumatoid arthritis and diarrhea of 5 mo. duration | 500 ml. | 4-5 | 8 | None |
| Pt. 5 (3A198), 55 kg., 65 yr., W., M.: Comatose following brain operation for metastases of hypernephroma | 300-500 ml. | 3 | 12 | If more than 0.8 Gm. fat/kg. body weight given, increase in respiratory rate and temperature elevation of 1 to 3° |
| Pt. 6 (7A320), 37 kg., 35 yr., W., F.: Bronchiectasis with malnutrition | 300-900 ml. | 4-5 | 6 | None |
| Pt. 7 (P1153), 45 kg., 68 yr., W., F.: Ovarian carcinoma with metastases | 300 ml. | 4-5 | 3 | None |
| Pt. 8 (9A857), 41 kg., 32 yr., W. F.: Chronic nephritis, uremia, hypertension, and pericarditis | 300 ml. | 3 | 3 | None |
| Pt. 9 (311320), 11 kg., 4 yr., W., F.: Mental defect with malnutrition | 100-300 ml. | 2 | 6 | If more than 3.5 Gm. fat/kg. body weight given, temperature elevation of 1 to 2° |
| Pt. 10 (A341898), 3.5 kg., 7 wk., W., M.: Postoperative obstruction | 50-150 ml. | 1-2 | 11 | None |
| Pt. 11 (341528), 15 kg, 6½ yr., W., M.: Ruptured appendix, peritonitis, and multiple abscesses | 300-500 ml. | 3 | 27 | If more than 4 Gm. fat/kg. body weight given, temperature elevation of 1 to 2° |

## CLINICAL OBSERVATIONS

Table I summarizes the data on the administration of fat emulsion to patients on the surgical and medical services of the Peter Bent Brigham Hospital and the Children's Hospital. The cases represent a wide variety of common ailments which result in weight loss of such an extent that provision for adequate calories becomes one of the major facets of good treatment (Table I). Only those patients who presented unusual findings with regard to the administration of the fat emulsion will be discussed and this discussion will be limited to that aspect of the cases.

*Patient 1.*—This patient was a 54-year-old, single, white woman weighing 37 kilograms admitted to the surgical service with the presenting complaint of anorexia and weight loss due to intractable epigastric pain aggravated by ingestion of food. There was a history of abdominal pain for thirty-three years and a total of eight abdominal operations. The patient appeared poorly nourished. On initial administration of fat, 300 ml. of emulsion were given in a period of sixty minutes without reaction. Thereafter, and for the next

three days, 500 ml. of emulsion furnishing approximately 800 calories were given daily with no subjective complaints or objective findings.

Before and during the administration of fat, observation of this patient's dietary habits revealed that she was a finicky eater and consumed an average of only about 1,200 calories per day. It was assumed that on such a caloric intake, this patient would be in negative nitrogen balance and would continue to lose weight, but that when this oral calorie intake was supplemented with 800 calories coming from fat emulsion the patient ought to be in positive nitrogen balance. This hypothesis was tested by doing a nitrogen and potassium balance study. The protein content of the 1,200 calorie oral diet was 45 Gm. and was provided mostly by animal protein. The potassium content of the daily diet

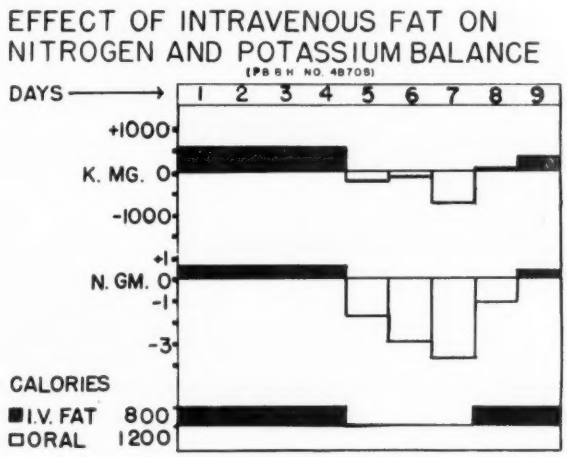


Fig. 1.

was 1.6 grams. Nitrogen determinations were all done by a macro-Kjeldahl method, and potassium was determined by the cobaltinitrite precipitation method.* Due to circumstances beyond our control, the periods with and without supplementary calories from the fat emulsion were not as long as desired. The results of this study are shown in Fig. 1. It is seen that during the first four-day period when the patient was receiving a total of 2,000 calories (1,200 calories orally which represented the average of the total daily caloric intake during hospitalization and 800 calories from fat given by vein) a positive potassium and nitrogen balance was obtained. During the three-day period when fat infusions were not given and the total caloric intake was only 1,200 calories, the patient promptly went into strong negative balance in both potassium and nitrogen. When fat infusions again were given, positive balances were obtained again. The nitrogen and potassium contributed by the emulsion were negligible.

*Patient 2.*—This patient was a 24-year-old white man weighing 40 kilograms with ulcerative colitis. An ileostomy had been done but did not function properly and the patient developed a peritoneal infection and a midjejunal fistula. It was necessary to reoperate to close the fistula. For several days prior to surgery, the patient's oral intake was nil and the parenteral therapy of blood, 5 per cent glucose, and electrolyte solution contributed no more than 400 calories per day. Because of small, sclerotic veins, 10 per cent glucose and protein hydrolysate preparations were not used. On the first postoperative day, 300 ml. of the fat emulsion were given. For ten of the next twelve days, daily infusions of fat emulsion varying from 600 to 900 ml. which furnished 960 to 1,440 calories were given. An exceptionally favorable clinical response followed the administration of the fat. At the end of this time oral intake of food was sufficient and all parenteral therapy was

*We are indebted to the Department of Surgery, Peter Bent Brigham Hospital, for doing the potassium determinations.

terminated. The caloric intake of this patient for the immediate postoperative period is shown in Fig. 2. It is seen that during the first three postoperative days, the fat emulsion contributed appreciably to the caloric intake; in fact, during the second and third postoperative days it furnished approximately 75 per cent of the total caloric intake. It was during this critical period that the favorable clinical response was especially noted. Because Emulsion 41 was not available, none was given on the fourth and fifth days. The data in Fig. 2 also emphasize quite strikingly the importance of ordinary food as a source of calories.

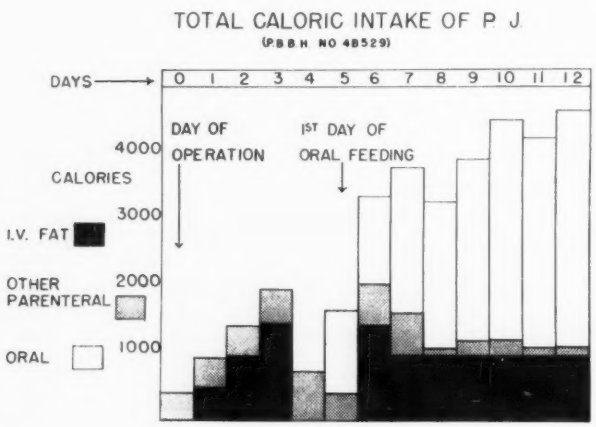


Fig. 2.

*Patient 9.*—This patient was a mentally defective child, 4 years of age, weighing 11 kilograms. Fat emulsion was given on six successive days in amounts varying from 100 to 300 ml., which furnished 30 to 60 per cent of the total calorie requirements. It was observed consistently that infusions of more than 250 ml. (3.5 Gm. fat per kilogram) at any one time produced an elevation of temperature of 1 to 2° which persisted for one to three hours following the fat infusion.

*Patient 10.*—This patient was a 7-week-old male infant, weighing 3 kilograms, with postoperative obstruction, who was on continuous gastric suction and complete parenteral feeding. The theoretical caloric requirement for this infant was approximately 300 calories and the usual parenteral fluids available could provide only approximately a third of this requirement. He was given fat emulsion by vein for eleven days, during which time he received on some occasions as much as 70 per cent of his basal caloric requirements from this source. Fig. 3 illustrates the caloric intake of this infant during a twelve-day period, the last nine days of which fat emulsion was given. Since the two preliminary small infusions of fat were well tolerated, the study presented in Fig. 3 was begun. The fat infusions were increased gradually in amount until by the third day they contributed more than one-half the total caloric intake (Fig. 3). On the fourth day and for the next five days, the theoretical caloric requirement of the infant was more than met, and calories from the emulsified fat furnished approximately two-thirds of the total caloric intake. Throughout this period the child was quite ill as a result of an extensive infection. Antibiotic therapy was ineffective and early in the morning of the thirteenth day of this study the child expired. Post-mortem studies gave no evidence that the extensive fat infusions, daily for nine days and on two other days shortly before the period represented by Fig. 3, had in any way contributed to the death.

*Patient 11.*—This patient was a 6½-year-old boy, weighing 15 kilograms, with a ruptured appendix, peritonitis, and multiple abscesses in whom complete parenteral nutrition was necessary. He received fat emulsion intravenously for twenty-seven days in quantities providing him with 480 to 800 calories per day which provided 50 per cent or more of his

daily calorie requirement. It was observed regularly in this patient that if more than 400 ml. of fat emulsion were given at any one time, there was a gradual rise of temperature of 1 to 2° during one to three hours following the infusion. This quantity of emulsion contained 60 Gm. of fat which furnished this patient with 4 Gm. of fat per kilogram. The infusions of fat in this child were clinically effective in that he presented a problem of complete parenteral feeding and further weight loss was prevented during the period fat infusions were given.

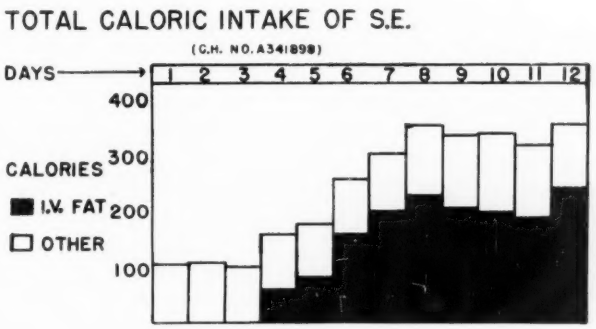


Fig. 3.

In view of the fact that an increase in temperature of 1 to 2° was consistently obtained in Patient 11 when the amount of fat given exceeded 4 Gm. per kilogram body weight, it was thought desirable to attempt to determine which component of the emulsion was largely responsible for the temperature rise. The following three different preparations were studied in Patient 11: the usual 15 per cent fat emulsion (Emulsion 41); a 15 per cent emulsion identical to Emulsion 41 except that the costabilizer of polyglycerol esters was reduced from 1 per cent concentration to 0.4 per cent; a fat-free emulsion which contained only the two stabilizers in the same concentration as in Emulsion 41 in 5 per cent dextrose. A different one of these three preparations was infused in Patient 11 in the same amount on each of three successive days. For the next three successive days these preparations were given in the same order, but the one without fat was given in twice the amount. The temperature rise occurred only when the amount of fat-containing emulsion infused exceeded 4 Gm. fat per kilogram of body weight.

That this temperature rise following infusion of a fat emulsion varies considerably among patients is suggested by the observations in Patient 9 in whom an amount exceeding 3.5 Gm. fat per kilogram of body weight resulted in a rise in temperature and in Patients 2 and 10 in whom as much as 3 and 6 Gm. fat per kilogram respectively did not give rise to an increase in temperature, yet it was the same batch of fat emulsion which was given to Patient 11. The data in Table I show that Patient 5, a 65-year-old man, weighing 55 kilograms, with cerebral metastases from a hypernephroma, and in whom most of the left frontal lobe had been removed, developed a temperature rise if more than 300 ml. of fat emulsion were given at any one time. This amounts to only 0.8 Gm. of fat per kilogram of body weight.

Insufficient evidence is available to indicate whether this rise in temperature is due to the amount of fat per se or to pyrogens contained in the fat which, when they reach a certain concentration, give rise to the increase in temperature. At present, the latter seems the more likely cause.

## DISCUSSION

The clinical observations presented in this paper indicate that fat emulsions as a source of calories in parenteral nutrition are useful in a wide variety of cases. Emulsion 41 furnished 1,600 calories per liter, and could be infused at the rates commonly employed for the intravenous administration of glucose or saline solutions.

Previous studies in the dog and rat have shown conclusively that such fat emulsions of fine particle size introduced directly into the systemic circulation are utilized for energy purposes as indicated indirectly by total carcass analysis for fat,[5] maintenance of normal weight,[5] conversion of negative to positive nitrogen balance on a low-protein low-calorie ration,[5] and growth of puppies,[6] and directly by radioisotope studies showing that $C^{14}$ introduced as part of the fat molecule in a fat emulsion is rapidly eliminated in the expired $CO_2$.[7] In previous studies with experimental animals and man, it has been shown that fat emulsions such as have been employed in the studies can be given safely and without producing pathologic conditions.[1, 3]

The studies reported in this paper extend many of these observations in man. The observations on Patient 1 confirm the favorable effect of fat emulsions administered intravenously on nitrogen balance. The similar observations on potassium balance further emphasize the necessity of adequate calories for protein synthesis in the body. The very favorable clinical response observed in the patient severely ill with ulcerative colitis (Patient 2) is attributed in part to the calories supplied by the fat emulsion during the first three postoperative days. In Patients 10 and 11, fat emulsions were given for rather long periods, eleven and twenty-seven days respectively, and in generous daily amounts, approximating 6 and 4 Gm. fat per kilogram of body weight. Patient 10, who was severely ill from a generalized infection, expired after nine successive days of fat infusion given in such an amount that calories from fat furnished two-thirds of the total caloric intake for a period of seven days. However, at autopsy, there was no abnormal accumulation of fat in any of the organs and no evidence that the fat infusions in any way had contributed to the death. During this severe illness, when all nourishment had to be given by vein, weight loss was prevented.

An increase in temperature in Patients 5, 9, and 11 was regularly observed following a single administration of a certain amount of fat. However, the amount of fat necessary to cause this temperature rise varied widely among these three patients—from 0.8 to 4 Gm. fat per kilogram of body weight. Patient 2, who received 3 Gm. fat per kilogram of body weight, and Patient 10, who received 6 Gm. fat per kilogram of body weight, showed no increase in temperature. It is thought that these temperature rises were due to pyrogenic materials in the fat and not to the fat per se.

Of the patients listed in Table I, three died by the time of the preparation of this paper—Patients 5, 8, and 10. Post-mortem examinations were obtained on the latter two and in neither was there any gross or microscopic pathologic condition attributable to the fat emulsions. Patient 5 expired approximately

one month following the last fat infusion; Patient 8, three months following the last infusion; and Patient 10, eighteen hours following the last infusion of fat.

It should be pointed out that the reason more fat emulsion was not given to many of these patients, and for longer periods of time, was that not enough was available because of limited production and control facilities and the needs of various experimental studies with animals. As stated in a previous paper[1] on this subject, Emulsion 41 is still not considered completely suitable for clinical use because of the development of pyrogenicity after it is approximately four weeks old.

## SUMMARY

Observations are reported on the intravenous administration of a 15 per cent fat emulsion to eleven patients representing a variety of common illnesses. Of these patients, eight were adults, two were children, and one was a 7-week-old infant. The emulsion furnished 1,600 calories per liter and was given at rates ordinarily used for administering glucose or saline solutions. Daily amounts up to 3 Gm. fat per kilogram of body weight were given to adults and 6 Gm. fat per kilogram of body weight in a 7-week-old infant. Infusion periods ranged from three to twenty-seven consecutive days. The emulsion was effective as indicated by favorable clinical response, the prevention of weight loss, and the maintenance of positive nitrogen and potassium balance. Subsequent post-mortem examination of three of these patients revealed that the fat emulsions had produced no pathologic changes, either gross or microscopic.

The authors wish to express appreciation to the Medical and Surgical Services of the Peter Bent Brigham Hospital and the Children's Hospital of Boston for their genuine cooperation in these studies, and to The Upjohn Company, Kalamazoo, Mich., which has supplied us generously with various materials used in this research.

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# THE ABSORPTION AND DISPOSITION OF ORALLY ADMINISTERED $I^{131}$-LABELED NEUTRAL FAT IN MAN

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NONE of the available methods for measuring the absorption and utilization of fat in man is greatly informative and, at the same time, accurate and simple to carry out. The direct chemical determinations of serum fat in serial fashion are tedious and time consuming; in the hands of all save the most expert they are subject to considerable inaccuracies. Relatively large samples are required. No information as to the disposition of the fat is available from this method, i.e., what proportions of the lipid which has disappeared from the blood stream are accounted for by degradation and by storage respectively. The vitamin A tolerance test obviates the first two of these objections, but not the third. Nephelometric techniques for fat analyses are now generally conceded to be unreliable.

The problem had been quite satisfactorily solved for animal work by Geyer and co-workers,[1] and Lerner and co-workers,[2] by the use of neutral fat containing radioactive $C^{14}$-substituted fatty acids. However, the extremely long half-life of this isotope precludes its use in man. In addition, other isotopes which emit radiation of greater penetrating power are more simple to measure.

Certain of the shortcomings mentioned may be overcome by the use of neutral fat which has been labeled with radioiodine $I^{131}$ in the unsaturated fatty acids. It is the purpose of this communication to report tracer studies with such radioactive fat on eighteen patients, including ten normal subjects.

## METHODS

Commercial olive oil was iodinated with $I^{131}$.* The following steps were employed. To the carrier-free $I^{131}$ solution (usually 10 mc.), 10 mg. of sodium iodide were added as carrier. The solution was then placed in a separatory funnel and acidified with 1 ml. of a 50 per cent concentrated nitric and sulfuric acid mixture. The liberated iodine was extracted with chloroform. Into the chloroform solution a stream of chlorine was then passed until the purple color of iodine just disappeared. The iodine chloride obtained was added to a chloroform solution of 20 Gm. of olive oil. This mixture was allowed to stand for twenty-four hours. The chloroform solution then was shaken repeatedly with a solution of sodium carbonate in order to remove any free iodine. The chloroform solution was dried over anhydrous sodium sulfate and the chloroform evaporated off by means of an infrared lamp. The odor, taste, color, and consistency of the oil were unchanged following iodination.

One-half to 5 ml. of the oil, containing 100 microcuries of $I^{131}$, were soaked into bread and eaten, either during or immediately following a normal breakfast. In a few instances the patients were without breakfast; all ate a normal mixed diet during the balance of the test period.

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This study was aided by grants from the United States Public Health Service, the Rockefeller Foundation, and the Godfrey H. Hyams Fund.

Received for publication, Aug. 30, 1949.

*By Mr. Charles Margnetti of Tracerlab, Inc., Boston, Mass.

The radioactivity in the thyroid gland was estimated by serial counting at a distance of 35 cm. from the neck with a sensitive, directionally shielded gamma tube (Sylvania). The "background" (from extrathyroidal tissues plus cosmic and other extraneous radiation) was calculated by counting over the lower thigh; this was subtracted from the thyroid count. The resulting net count was compared with that obtained under similar geometric conditions from a suitable aliquot of the original solution.

One milliliter portions of urine in uniform glass vials were counted with the gamma tube through an orifice in the shield and compared with equal volumes of suitable dilutions of the original solution. Since the bottoms of the vials were in contact with the metal tube surface, excellent reproducibility and sensitivity (272 net counts per second per microcurie) were obtained.

Two-tenth milliliter aliquots of serum were pipetted onto flamed, 1 inch copper planchets, each of which was partially covered with a smaller disk of thin absorbent paper. Since the planchets were grease-free and were coated with cupric oxide, the liquid was absorbed uniformly into the paper. After slow drying with an infrared lamp, the serum formed an evenly adherent film, the area of which was limited to that of the paper circle. The preparations were permanently mounted by covering with Scotch tape. Standards were prepared by evaporating suitable aliquots of petroleum ether solutions of the original material in the same manner; self-absorption was equalized by adding identical amounts of serum. The samples were counted with a thin-window Geiger-Müller tube.

Two milliliters of serum were diluted, precipitated with zinc sulfate, and the excess zinc precipitated with alkali by the method of Somogyi. Five milliliter portions of the supernatant (equivalent to 0.5 ml. serum) were evaporated onto disks and the radioactivity was compared with similarly prepared standards as described. Since the lipids were precipitated with the proteins, the radioactivity in the watery fraction of the serum represented inorganic iodine which had been removed from the fat. By subtraction of this "water-soluble" portion from the total, a fraction designated as the "lipid $I^{131}$" was obtained.

In seven patients the sera were separated by means of Bloor's solution and the radioactivity was determined in the lipid and water-soluble fractions of this extract. There was, in general, agreement between the results obtained by this procedure and the former one. However, there was usually slightly more radioactivity in the portion resulting from petroleum ether extraction of the dried Bloor's extract than when this lipid fraction was calculated by subtraction. Because of the greater simplicity of the zinc precipitation method, it was used exclusively later.

## RESULTS

In ten normal subjects the pattern of absorption and disposition was constant. The concentrations of total $I^{131}$ in the serum were greatest at three to six hours following the meal and slowly decreased after this time. The peaks in both the "total $I^{131}$" and "lipid $I^{131}$" curves occurred simultaneously (Fig. 1). These levels of total radioactivity ranged from 0.4 per cent to 0.6 per cent per 100 ml. of serum, whereas the highest concentrations of the "lipid" fractions varied from 0.20 per cent to 0.47 per cent per 100 ml. of serum.

The accumulation of radioactivity by the thyroid gland varied from 10 to 25 per cent in twenty-four hours (Fig. 1). Although appreciable, this was somewhat less than the average collection of a dose of *inorganic* $I^{131}$ administered for the purpose of ascertaining thyroid function.[3] Likewise, the excretion in the urine of 15 to 48 per cent of the ingested radioiodine by the normal controls in twenty-four hours was distinctly less than if the material administered had been inorganic iodine.

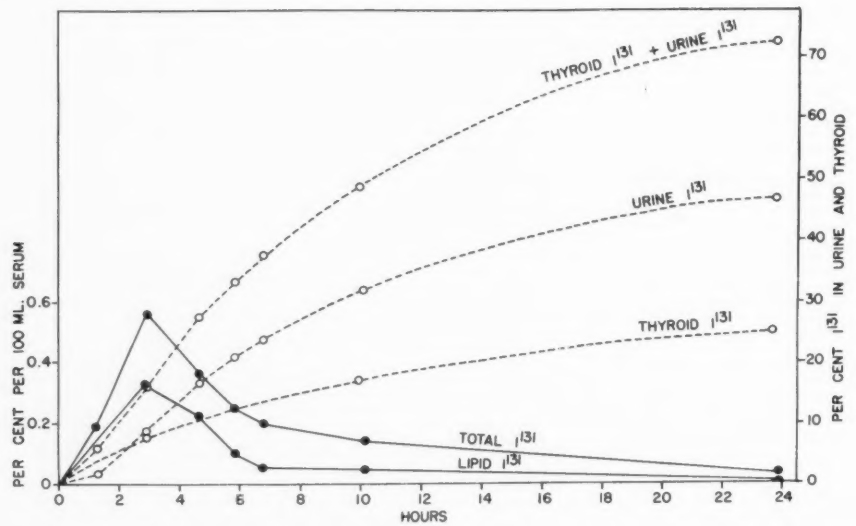


Fig. 1.—Curves showing the fate in a normal subject of radioiodine introduced into the body as labeled fat. The time in hours following the ingestion of the iodinated fat is plotted on the abscissa. Along the left ordinate are plotted the concentrations of radioactivity attained on the serum; variations in these values are indicated by the solid lines. The broken lines show the percentage of administered radioactivity which was excreted in the urine and collected by the thyroid (right vertical scale).

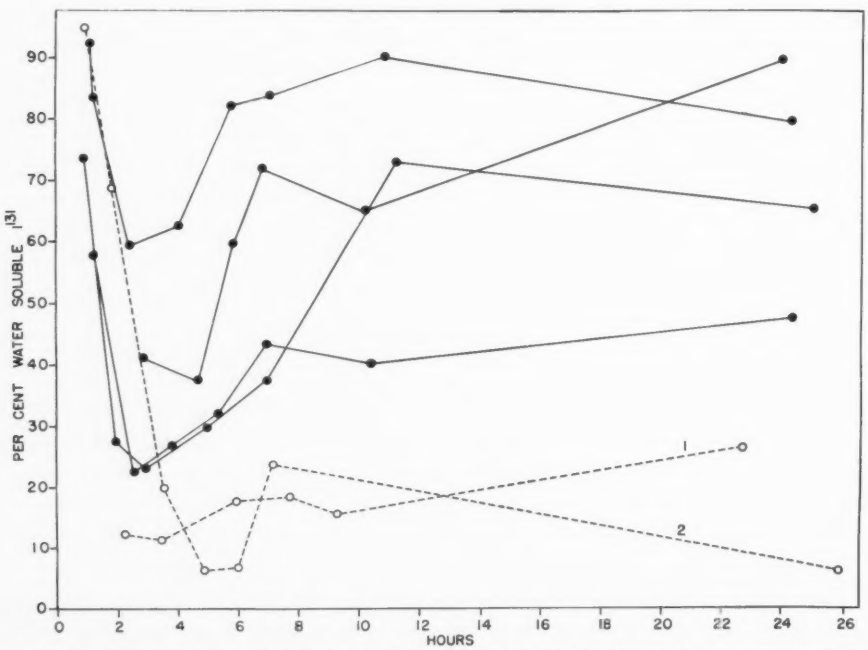


Fig. 2.—The variation with time of the fraction of radioactivity in the "water soluble" portion of the serum. The sera were fractionated as described in the text; the "water-soluble $I^{131}$" is plotted as per cent of the total radioactivity. The solid lines and circles show these values in four normal subjects while the broken lines and open circles denote a patient with idiopathic hyperlipemia (*2*) and the nephrotic syndrome (*1*). In the normal subjects (and in the patient with idiopathic hyperlipemia [*2*]) during the first hour of the test 60 to 90 per cent of the radioactivity was in the water-soluble fraction. In the normal subjects this sharply decreased during the next one to two hours, as absorption of the iodinized olive oil reached a maximum, and returned slowly to the high values during the next four to seven hours. In the hyperlipemic patients the percentage of radioactivity in the water-soluble portion of the serum remained low throughout the rest of the test after the first two hours.

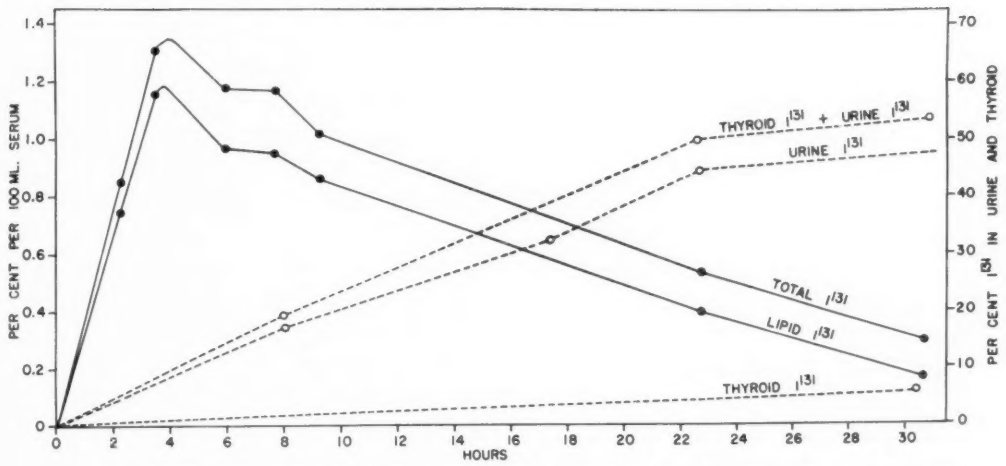


Fig. 3.—The absorption and disposition of labeled fat in a patient with the nephrotic syndrome. The data are indicated as in Fig. 1. It will be seen that the concentrations of radioactivity in the serum, principally in the lipid fraction, are much greater than normal; the decrease in these high levels is abnormally slow. The excretion of $I^{131}$ in the urine and its accumulation in the thyroid, especially during the early hours of the test, are also somewhat slower than normal.

The changes in the ratio of serum inorganic to "lipid" iodine also were uniform (Fig. 2). During the first one to two hours from 60 to 90 per cent of the $I^{131}$ was in the water-soluble portion. Later, as absorption of the iodinized olive oil progressed the size of the inorganic fraction decreased sharply, then gradually returned to the high values during the ensuing eighteen hours.

In three patients with high serum neutral fat levels (two subjects with "idiopathic" hyperlipemia and one individual with the nephrotic syndrome) the peak "lipid $I^{131}$" values were two to five times normal.[4] There was an abnormally slow decrease in the radioactivity during the twenty-four hours following these high values. A larger than normal portion of the radioiodine remained in the lipid fraction (Fig. 2), and the accumulation of radioactivity in the thyroid and the excretion of the radioiodine in the urine were abnormally slow also (Fig. 3). In one patient with hypercholesterolemic xanthomatosis the test was normal. In one case of sprue the highest serum concentrations of total $I^{131}$ were about one-half normal. The urinary excretion and thyroid collection were correspondingly low, but there was a normal relationship between the inorganic and "lipid" fractions in the serum at all times.

## DISCUSSION

It should be emphasized that the procedure under discussion was not a "tolerance test" in the usual meaning of the term. The amount of oil administered was never more than 5 Gm.; this served to label the fat being absorbed from the meal eaten with the tracer, as well as that already in the blood stream.

Olive oil contains about 80 per cent glycerol trioleate and about 7 per cent trilinoleate. The former of these 18-carbon fatty acids possesses one double bond between the 9th and 10th carbon atoms, while the latter has two such

bonds. The saturated compounds tristearate and tripalmitate comprise the bulk of the remainder of the lipids of the oil.

As a result of iodination the radioiodine was firmly attached at the double bonds in the unsaturated fatty acid chains; the process used was similar to the familiar method for determination of the "iodine number" of fats. In order to be certain that this iodine was organically bound, petroleum ether solutions of the oil in each lot were repeatedly extracted with water; no radioactivity was detectable in the aqueous phase.

It may be accepted as proved that the iodine collected by the thyroid gland is in the form of the iodide ion.[5] The identity of the iodine excreted in the urine by the subjects in this study has not been determined, although it is entirely likely that it is in the same form. The radioactivity in the water-soluble portion of the serum and that collected by the thyroid and excreted in the urine represent iodine which has been removed from the fatty acid skeleton during the process of metabolism; these fractions provide an index of the rate of utilization of the iodinated fat.

In this study, from 50 to 73 per cent of the administered radioactivity was collected in the thyroid gland and excreted in the urine of normal subjects during the twenty-four hours from the beginning of the test, with approximately one-half these amounts accounted for during the first six hours. These figures indicate the extent of degradation of the fatty acid skeleton of ingested neutral fat which normally occurs in men, either as a preliminary to storage in the fat depots or, much more likely, for production of energy. The characteristic appearance of an appreciable portion of radioactivity in the water-soluble fraction of the serum during the early hours of the test suggests that a small amount of breakdown may have occurred during the process of absorption from the gastrointestinal tract.

That a similarly rapid metabolism of intravenously administered neutral fat takes place in the rat has been recently demonstrated by Geyer and co-workers[1] and by Lerner and associates.[2] The latter group used a 16-carbon fatty acid (as tripalmitin) with the 6th carbon atom labeled with $C^{14}$; as much as 59 per cent of the injected $C^{14}$ was found in the expired air in twenty-four hours. The type of fat administered and the position of the labeled atom were not identical in the experiment of Lerner and co-workers and in the present study. However, the curves representing the sum of urinary excretion plus thyroid accumulation of $I^{131}$ in the normal subjects in this study closely resemble those depicting the cumulative excretion of $C^{14}O_2$ by the rats in the experiment of Lerner, Chaikoff, Entenman, and Dauben.

## SUMMARY

Characteristic curves of absorption and utilization of physiologic amounts of lipid taken by mouth by subjects on a mixed diet were demonstrated by the use of unsaturated fats iodinated with $I^{131}$. The proportion of the administered radioactive iodine which was collected by the thyroid and excreted in the urine, as well as the magnitude of the water-soluble portion in the serum, indicated

the extent of breakdown of the labeled fat. Under these circumstances in normal subjects, degradation of from 50 to 73 per cent of the orally administered iodinated fat took place within twenty-four hours. Subjects with "idiopathic" hyperlipemia and the nephrotic syndrome utilized the labeled lipid much more slowly.

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# THE EFFECT OF SPLENECTOMY ON THE TOXICITY OF $SR^{89}$ TO THE HEMATOPOIETIC SYSTEM OF MICE

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## INTRODUCTION

RADIOSTRONTIUM ($Sr^{89}$), a $\beta$-ray emitter with a fifty-five day half-life, which is physiologically interchangeable with calcium, localizes largely in bone soon after enteral or parenteral administration.[1-6] The minimum intraperitoneally administered dose required to produce a significant leucopenia in CF-1 female mice* was found by Simmons and Jacobson[7] to be about 0.068 microcuries per gram of body weight. However, this leucopenia was relatively transient. Brues and co-workers[8] have reported that bone sarcomas are readily produced in this strain with this dose. Although a severe and persistent leucopenia developed after an intraperitoneal dose of 2.0 microcuries per gram of body weight, no significant anemia occurred.[7] In a preliminary experiment[9] histopathologic examination of mice sacrificed at intervals after injection revealed much depleted or aplastic bone marrow, whereas the spleens contained a remarkable increase in erythrocytopoiesis. The fact that anemia failed to develop under these circumstances was considered to be directly related to the rapid development of an intense ectopic erythrocytopoiesis in the spleens of the radiostrontium-treated animals. Accordingly, an experiment of greater scope, as described in this communication, was undertaken to determine the validity of this concept.

## MATERIALS AND METHODS

Young CF-1 female mice were divided into three groups of fifty or more animals each as shown in Table I. The mice in Group I were splenectomized; those in Group II received a single intraperitoneal injection of 2.0 microcuries of $Sr^{89}$ per gram of body weight, and the mice in Group III were splenectomized and later injected with a dose of 2.0 microcuries per gram of body weight. A fourth group of animals not included in Table I, which was neither splenectomized nor given $Sr^{89}$, was used for base line hematologic controls and from this group animals were sacrificed at intervals to serve as normal reference for the histologic studies. Splenectomies were performed in Groups I and III while the animals were under ether anesthesia. Twenty-four days after splenectomy, $Sr^{89}$ was administered intraperitoneally to the animals in Group III. The average weight of the mice at the time of injection was 20 grams. A second injection of 2.0 microcuries per gram was given to a representative number of animals in Groups II and III, 119 days after the original injection. Although a leucopenia of moderate degree persisted at this time in both groups (II and III), the animals in Group II which had been subjected to splenectomy and received $Sr^{89}$ also had

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The photomicrographs were made by Jean M. Crunelle of the Photographic Department, Billings Hospital, University of Chicago.
Received for publication, Sept. 2, 1949.
*Raised by Carworth Farms; homozygous for aa bb cc.

TABLE I. GENERAL SCHEMATA OF EXPERIMENT

| GROUP | NUMBER OF ANIMALS | TREATMENT | PERIOD OF OBSERVATION AFTER ORIGINAL $Sr^{89}$ INJECTION |
|---|---|---|---|
| I | 51 | Splenectomy only | 282 days |
| II* | 63 | Injected intraperitoneally with $Sr^{89}$ in a dose of 2.0 microcuries per gram body weight | 282 days |
| III | 70 | Splenectomy plus $Sr^{89}$ (2.0 microcuries per gram body weight) | 282 days |

*Selected animals in Groups II and III were reinjected with a dose of 2.0 microcuries per gram body weight 119 days after original injection.

recovered from the anemia. The purpose of the second injection of $Sr^{89}$ was largely to determine whether or not compensatory ectopic erythrocytopoiesis was sufficient in the splenectomized mice to prevent or minimize recurrence of anemia.

*Preparation of Radiostrontium ($Sr^{89}$): Distribution and Excretion Studies.*—As has been described elsewhere,[5] the solution of radiostrontium was composed of $Sr^{89}$ and $Sr^{90}$. $Sr^{89}$ has a fifty-five day half-life and a maximum energy of 1.7 mev; $Sr^{90}$, which represented 3 to 5 per cent of the initial stock solution on arrival from Oak Ridge, has a half-life of circa thirty years and a maximum energy of 0.65 mev. The final preparation for administration was strontium chloride in isotonic solution. The greatest part of the radiostrontium excretion occurred during the first three days, and after ten to fifteen days virtually no more of the retained $Sr^{89}$ was excreted. The average total excretion values were 54 per cent of the injected dose. Average total retention values were 45 per cent of the injected dose. By the third day after injection almost all of the retained $Sr^{89}$ was deposited in the skeleton. Retention of $Sr^{89}$ in the spleen was as follows:

| | |
|---|---|
| First 24 hours | 0.22 per cent/gram |
| 24 to 72 hours | 0.04 per cent/gram |
| 3 days to 3 months | 0.02 per cent/gram |

In other soft tissues of the body, the amount of radiostrontium per gram remaining after three days was well under 0.1 per cent of the injected dose.

*Hematologic Studies.*—Studies of the peripheral blood were made on all four groups. These studies included determinations of the hemoglobin in grams per 100 ml. (photoelectric), erythrocytes and leucocytes per cubic millimeter, leucocyte differentials (Wright's stain), and reticulocytes per cubic millimeter and in per cent (brilliant cresyl blue). Blood for study was drawn from the tail vein.

Control hematologic determinations were made on all animals after which splenectomy was performed on animals in Group I and III. Twenty-four days after splenectomy, $Sr^{89}$ was injected intraperitoneally into the animals in Groups II and III. Repeat hematologic determinations were made on all animals seven days after the original $Sr^{89}$ injection and thereafter at twenty-one day intervals. After the second injection of $Sr^{89}$ in selected animals, hematologic studies were made at less frequent intervals.

*Histologic Studies.*—Animals prepared as described were sacrificed at intervals through 280 days after $Sr^{89}$ injection for histopathologic study. In addition, random animals in all groups on which hematologic studies were being conducted were sacrificed at various intervals. This latter procedure was followed in an attempt to correlate more closely the peripheral blood studies with the actual histopathologic state of the blood-forming tissue.

Tissues taken for study included skin, lung, small intestine (with Peyer's patch), adrenal, kidney, liver, spleen, thymus, lymph node, and bone marrow (femur and vertebrae). The tissues were fixed in Zenker-formol, embedded in 20 per cent nitrocellulose, sectioned at 8 $\mu$, and stained with hematoxylin-eosin-azure II.

RESULTS

*Hematologic Studies on the Peripheral Blood.—*

*(a) Effect of Splenectomy Alone:* The mean leucocyte value in the peripheral blood of splenectomized mice rose after splenectomy to stabilize between 14,000 and 22,000 per cubic millimeter during the period of observation (Fig. 3). Splenectomy alone reduced the hemoglobin and erythrocyte values slightly during the first three weeks after splenectomy, but thereafter, as is shown in Figs. 1 and 2, no appreciable fluctuation occurred.

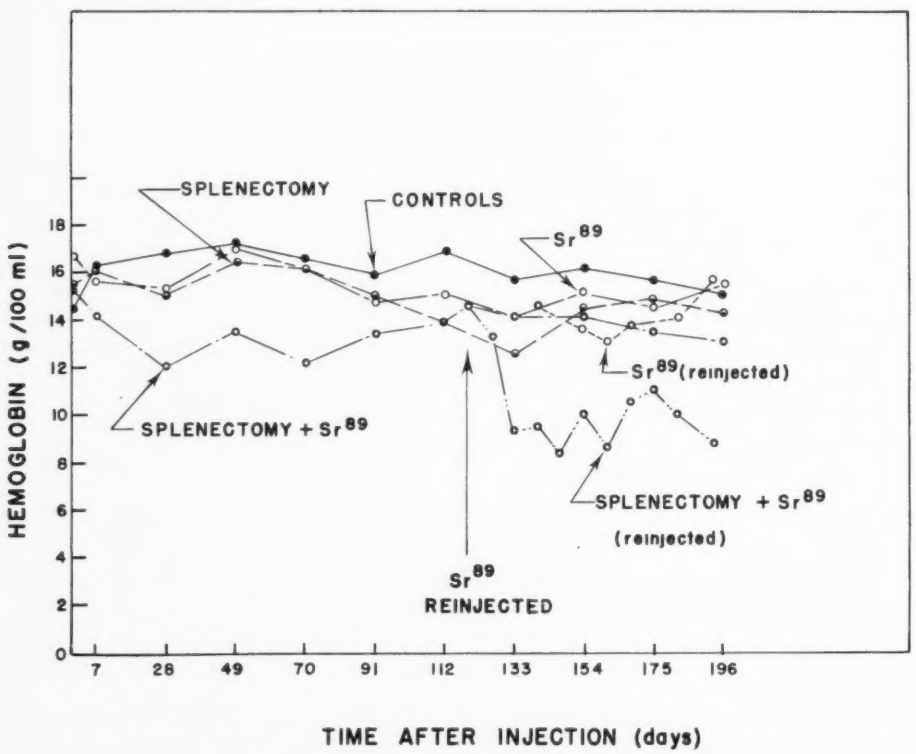


Fig. 1.—The effect of a single injection of 2.0 microcuries of radiostrontium per gram body weight on hemoglobin values of normal mice and mice splenectomized twenty-four days prior to $Sr^{89}$. One hundred nineteen days after the original $Sr^{89}$ injection, a second injection of 2 microcuries per gram body weight was given to selected animals from the two groups which originally were injected with this isotope.

*(b) Effect of $Sr^{89}$ Injection on Leucocytes of Peripheral Blood:* The intraperitoneal injection of 2.0 microcuries of $Sr^{89}$ per gram produced a comparable degree of leucopenia in animals with an intact spleen and in animals that previously had a splenectomy (Fig. 5). As is shown in Figs. 4 and 5, the initial injection of $Sr^{89}$ reduced heterophils and lymphocyte values by 52 and 36 per cent, respectively, in twenty-eight days. The mean heterophil value of $Sr^{89}$-injected animals which had intact spleens returned to the preinjection level in about forty-nine days and thereafter was actually higher than the

control value. The mean heterophil value of animals which had been splenectomized and were given $Sr^{89}$ returned to the normal control or preinjection level in seventy days. The lymphocyte reduction after $Sr^{89}$ injection was sustained in the animals with intact spleens and in splenectomized animals. In animals to which a second injection of $Sr^{89}$ was given 119 days after the first injection, the reduction in heterophil and lymphocyte values was greater and more persistent in the splenectomized mice than in the intact mice (Figs. 4 and 5).

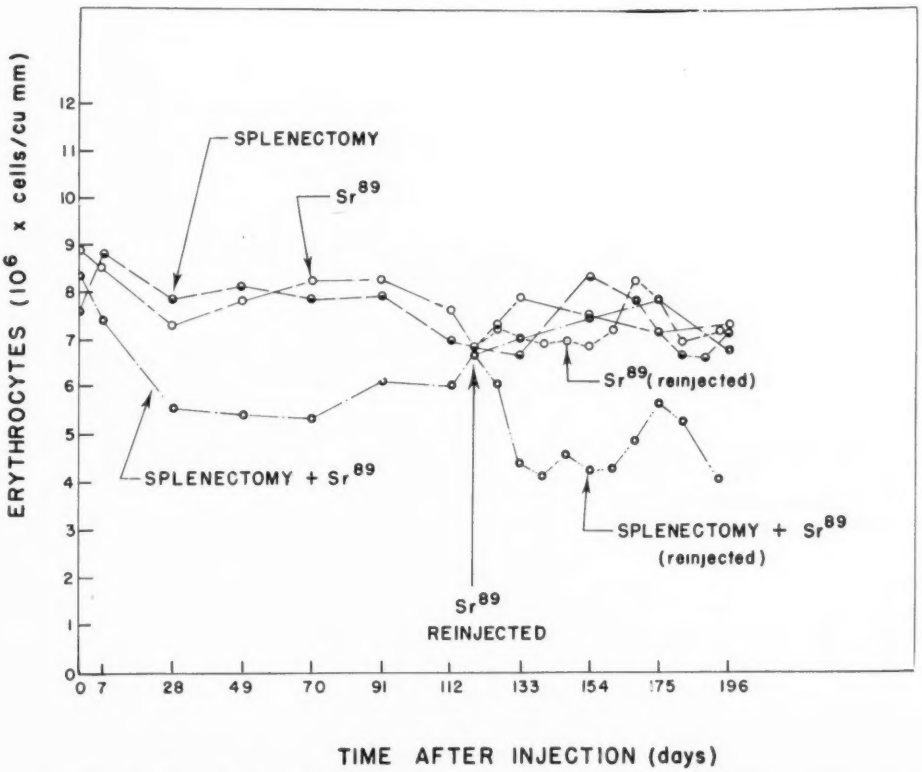


Fig. 2.—The effect of a single injection of 2.0 microcuries of radiostrontium per gram body weight on erythrocyte values of normal mice and mice splenectomized twenty-four prior to $Sr^{89}$. One hundred nineteen days after the original $Sr^{89}$ injection, a second injection of 2 microcuries per gram body weight was given to selected animals from the two groups which originally were injected with this isotope.

*(c) Effect of $Sr^{89}$ Injection on the Hemoglobin and Erythrocyte Values of the Peripheral Blood:* No anemia of significance was produced in mice with intact spleens to which $Sr^{89}$ was given by intraperitoneal injection. On the other hand, splenectomized mice injected with 2.0 microcuries per gram developed a significant anemia in twenty-eight days which persisted through seventy days, but with recovery in 119 days (Figs. 1 and 2). Selected animals with an intact spleen and animals which had been splenectomized were given a second injection of $Sr^{89}$ (2.0 microcuries per gram) 119 days after the

original injection. As after the initial $Sr^{89}$ injection, the mice with intact spleens developed no anemia, whereas the splenectomized group developed a precipitous reduction in both hemoglobin and erythrocyte values. Although recovery from the anemia proceeded in the splenectomized group, the anemia recurred between the fifty-sixth and seventy-seventh day after the second injection at which time all the remaining animals died or were sacrificed for study (Figs. 1 and 2).

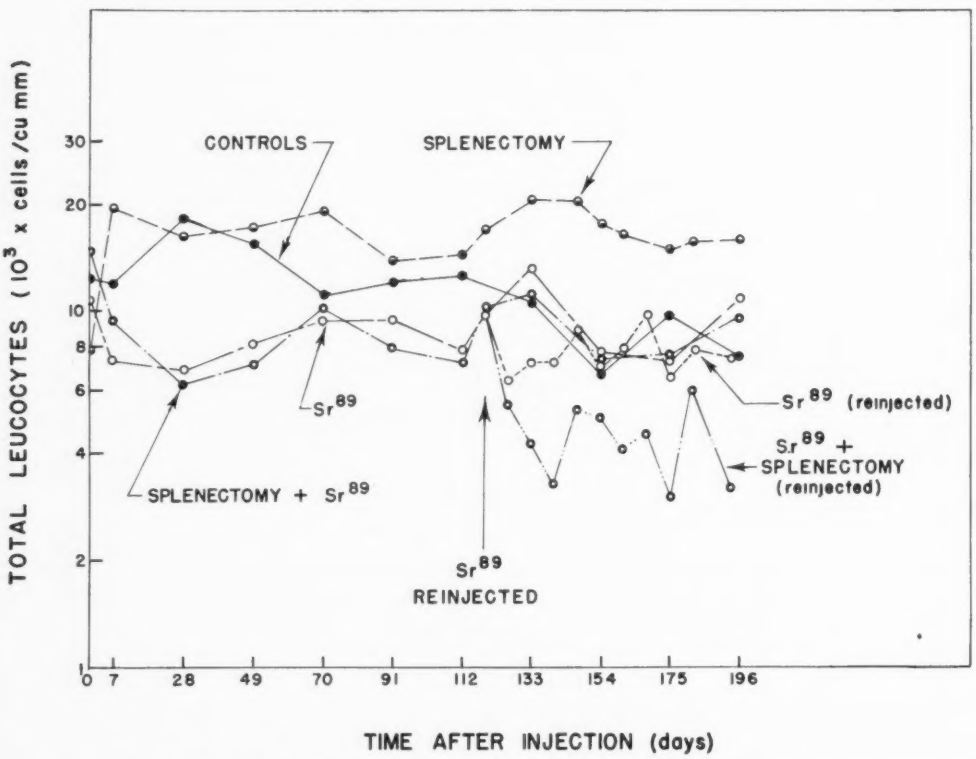


Fig. 3.—The effect of a single injection of 2.0 microcuries of radiostrontium per gram body weight on leucocyte values of normal mice and mice splenectomized twenty-four days prior to $Sr^{89}$. One hundred nineteen days after the original $Sr^{89}$ injection, a second injection of 2 microcuries per gram body weight was given to selected animals from the two groups which originally were injected with this isotope.

*(d) Effect of $Sr^{89}$ on Reticulocyte Values of the Peripheral Blood:* Except for control studies no reticulocyte determinations were made until seven days after $Sr^{89}$ injection; but at this point the reticulocyte values of animals which were splenectomized only and those which were given $Sr^{89}$ only had actually risen, whereas the reticulocyte value of the group which was both splenectomized and strontium-treated had fallen significantly. Reinjection of $Sr^{89}$ at 119 days produced no reduction in the reticulocyte value of mice with intact spleens. A reduction comparable with that seen after the original injection occurred; however, this was limited to splenectomized mice. As will be evident in the section on histology which follows, more frequent determinations

of the reticulocyte values of all the groups would have been of importance from the point of view of correlation.

*Histopathologic Studies.—*

*(a) Observations on Lymphatic Tissue After Single Injection of $Sr^{89}$:* No consistent difference in size of the spleens existed between $Sr^{89}$-injected and control mice which were sacrificed during the 280-day period. However, an effect was observed in both the white and red pulp of the spleen in animals sacrificed three days after $Sr^{89}$ treatment. This early effect consisted of a decreased cellularity of the white pulp and a marked hyperplasia of ectopic

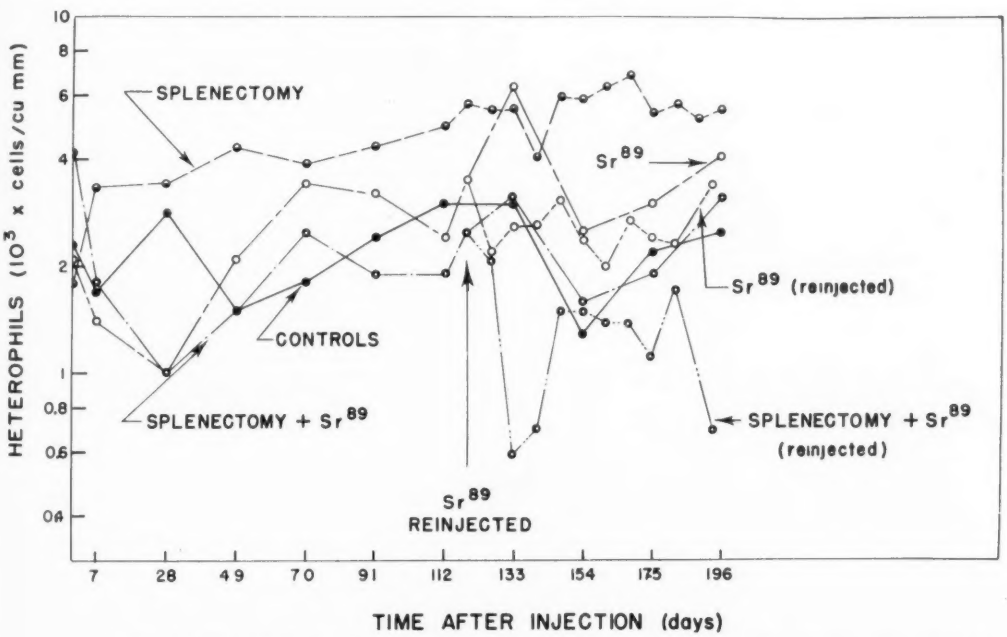


Fig. 4.—The effect of a single injection of 2.0 microcuries of radiostrontium per gram body weight on heterophil values of normal mice and mice splenectomized twenty-four days prior to $Sr^{89}$. One hundred nineteen days after the original $Sr^{89}$ injection, a second injection of 2 microcuries per gram body weight was given to selected animals from the two groups which originally were injected with this isotope.

erythrocytopoiesis and megakaryocytopoiesis in the red pulp; ectopic granulocytopoiesis was practically absent at this stage. Only a few scattered mature granulocytes could be found. There was little evidence of destructive effects, however, for there was no observable increase in pigment or debris-filled macrophages or pyknotic nuclei at this stage as compared with the spleens of control mice. The spleens of control mice usually contained small foci of ectopic erythrocytopoiesis, granulocytopoiesis, and megakaryocytopoiesis scattered about in the red pulp. The erythrocytopoiesis in the spleens of the $Sr^{89}$-injected animals, however, was diffuse and intense at three days and consisted of predominantly large hemocytoblasts, basophilic erythroblasts, and a lesser number of more mature forms.

The number of young megakaryocytes in the spleens of $Sr^{89}$-injected mice three days after injection was greatly above that of the controls. This intense erythrocytopoiesis and megakaryocytopoiesis persisted in the $Sr^{89}$-injected animals at all the periods of examination through 280 days. Photomicrographs of the spleens of $Sr^{89}$-injected and control mice at intervals of three, twenty-nine, forty-five, and ninety-two days (Fig. 6) illustrate especially the

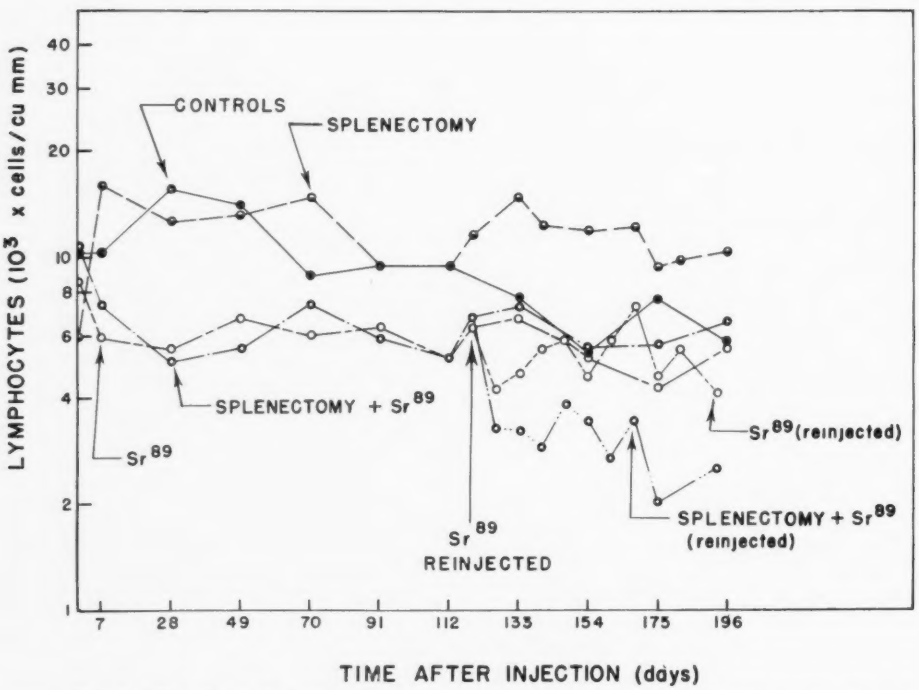


Fig. 5.—The effect of a single injection of 2.0 microcuries of radiostrontium per gram body weight on lymphocyte values of normal mice and mice splenectomized twenty-four days prior to $Sr^{89}$. One hundred nineteen days after the original $Sr^{89}$ injection, a second injection of 2 microcuries per gram body weight was given to selected animals from the two groups which originally were injected with this isotope.

degree of erythrocytopoiesis at these stages. Fig. 7 illustrates the closely packed erythroblasts in the red pulp at some of these stages at a slightly greater magnification.

No increase in ectopic granulocytopoiesis was observed in spleens of mice sacrificed at intervals of three and eight days after injection. Increased granulocytopoiesis became apparent in twelve days and increased to a maximum between forty-five and ninety-two days. In fact, in several of the mice examined during the forty-fifth and ninety-second days, granulocytopoiesis was qualitatively as intense as erythrocytopoiesis.

Changes in the white pulp in the spleens of $Sr^{89}$-treated mice were more or less universal at all stages studied from 3 to 280 days after injection. In normal mice the white pulp occupies circa 50 per cent of the spleen and, except for the distinct lymphatic nodules, blends gradually into the red pulp.

The splenic white pulp of the $Sr^{89}$-injected mice almost without exception occupied less space than the red pulp and was distinctly separated from the intense erythrocytopoietic and granulocytopoietic red pulp by a zone of relative acellularity consisting largely of reticular cells (Figs. 6 and 7). The decrease in cellularity of the white pulp was observed especially in the early

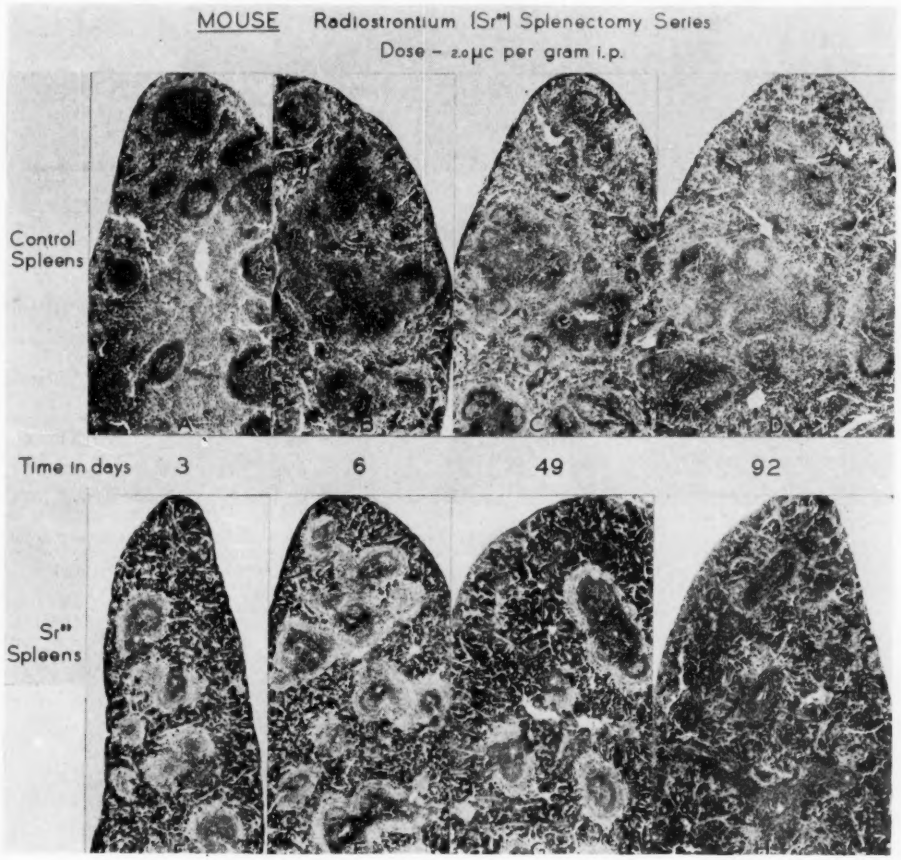


Fig. 6.—Photomicrographs of sections of the spleens of mice at intervals after $Sr^{89}$ injection. The normal spleens show small areas of ectopic hematopoiesis in the red pulp and the normal variation in the white pulp, whereas the spleens of $Sr^{89}$-injected animals show an intense ectopic hematopoiesis in the red pulp and a depletion of the cellularity of the white pulp. (Hematoxylin-eosin-azure II, ×15.)

stages after injection (i.e., three, six, twelve, fifteen, twenty-one, and twenty-nine days), but the atrophy, which persisted, is illustrated in the photomicrograph of the ninety-second day specimen (Fig. 7).

*(b) Lymph Nodes, Peyer's Patches of Intestine, and Thymus:* Lymph nodes from the root of the mesentery and Peyer's patches from the small intestine from all animals sacrificed were studied in control and $Sr^{89}$-injected groups. As opposed to the white pulp of the spleen of $Sr^{89}$-injected animals in which atrophy, reduced cellularity, and absence or diminution in the number of active lymphatic germinal centers were observed, the lymph nodes and

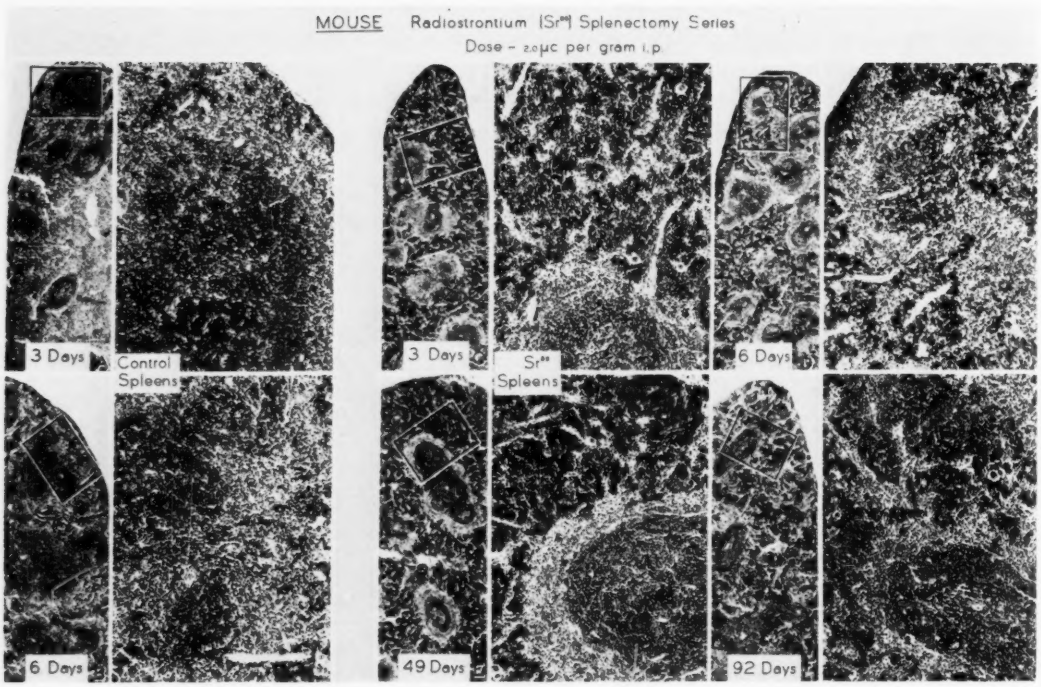


Fig. 7.—Photomicrographs of normal spleens and the spleens of $Sr^{89}$-injected animals with selected areas at a higher power to illustrate the intensity of the ectopic hematopoiesis in the red pulp and the depletion of cellularity in the white pulp of $Sr^{89}$-injected animals. (Hematoxylin-eosin-azure II; low power ×15, high power ×70.)

Peyer's patches appeared essentially normal and the germinal centers were active. Granulocytopoiesis, erythrocytopoiesis, and megakaryocytopoiesis were definitely increased in the medulla of the lymph nodes of some of the $Sr^{89}$-injected mice as compared with control mice. This increase in ectopic hematopoiesis in lymph nodes was observed as early as fifteen days after injection but was more prominent in later stages. No increase or decrease in plasma cells in lymphatic tissue was observed. No histologic changes which could be directly attributed to $Sr^{89}$ were observed in the thymus.

*(c) Observations on Lymphatic Tissue After Second Injection of $Sr^{89}$ at 119 Days:* Only a relatively few stages were studied. These were at fifteen, twenty-four, and seventy-seven days after the second injection.

The histologic effects as far as lymphatic tissues were concerned were again limited to the spleen. The lymph nodes, Peyer's patches, and the thymus appeared essentially normal except for a moderate increase in granulocytopoiesis, megakaryocytopoiesis, and erythrocytopoiesis in the lymph nodes. At the time of the second injection, the erythrocyte and hemoglobin values were essentially normal in both groups of mice which had originally received $Sr^{89}$ (Figs. 1 and 2). The marked ectopic erythrocytopoiesis, megakaryocytopoiesis, and granulocytopoiesis, however, persisted in the spleens of the $Sr^{89}$-injected animals sacrificed at 119 days at a time comparable with

that at which selected animals received a second $Sr^{89}$ injection. Although moderate atrophy of the white pulp was also apparent at this time, active lymphatic germinal centers were present and, in general, the white pulp was more cellular than in earlier stages.

By the fifteenth day following the second injection of $Sr^{89}$, ectopic erythrocytopoiesis, megakaryocytopoiesis, and granulocytopoiesis were more intense than at the 119-day stage. Ectopic granulocytopoiesis, however, was only moderate in degree. Again, as after the first injection of $Sr^{89}$, little evidence existed of active cellular disintegration in the form of pyknosis, karyorrhexis, or phagocytosis. The white pulp, which showed recovery to some extent after the original injection of $Sr^{89}$ at 92 and 119 days in that it constituted a more normal percentage of the splenic tissue and was richer in medium and small lymphocytes, again was reduced in amount with a depletion of lymphocytes in the periphery of the white pulp fifteen days after the second injection. In the two other stages studied following the second injection, namely twenty-four and seventy-seven days, the recovery of the white pulp was only moderate; atrophy and decreased cellularity persisted.

As was true after the initial injection of $Sr^{89}$, essentially "maximum" increased ectopic erythrocytopoiesis and megakaryocytopoiesis were apparent fifteen days after the second; granulocytopoiesis was progressively more intense at the twenty-four and seventy-seven day stages. Pigment-filled macrophages, scattered throughout the red pulp, were increased in number at all stages studied after the second injection.

*(d) Observations on Bone Marrow After a Single $Sr^{89}$ Injection:* The observations made on bone and bone marrow are limited to the effects of $Sr^{89}$ on hematopoiesis in the marrow spaces. A vertebra and a femur were taken for study from each animal sacrificed. Since the effects of $Sr^{89}$ on vertebral or femoral marrow were essentially comparable in the mice with intact spleens and in those that were splenectomized prior to the injection, no attempt to differentiate between the two groups will be made in the description of marrow changes. In general, the destructive effects of $Sr^{89}$ on hematopoiesis were comparable in femoral and vertebral marrow in that if complete or partial atrophy were found in the entire femur, the condition was also present in the vertebral bone marrow and vice versa. However, moderate decrease in cellularity with or without fibrosis was not infrequently found in the metaphyseal region of the femur immediately adjacent to the epiphyseal cartilage without evidence of such localized changes in the vertebral marrow. The destructive effects of $Sr^{89}$ and the subsequent depletion of the normal hematopoietic cells were most severe in the metaphyseal region, of equal or slightly less severity in the epiphyseal marrow, and least in the diaphysis of the femur. In some instances essentially complete depletion of normal hematopoiesis was found in the entire femoral and vertebral marrow spaces of mice sacrificed at fifteen through eighty days. In others, although general cellularity of the femoral diaphysis and vertebral marrow space was reduced, complete depletion was present only in the metaphyseal and epiphyseal regions of the bone.

In spite of the cellular depletion that occurred after $Sr^{89}$ injection, no stage studied either in femoral or vertebral marrow showed clear-cut histologic evidence of increased death of cells. The number of pyknotic nuclei and macrophages filled with particulate debris did not exceed that that was observed in control mice. A marked depletion of cells in the epiphyseal and

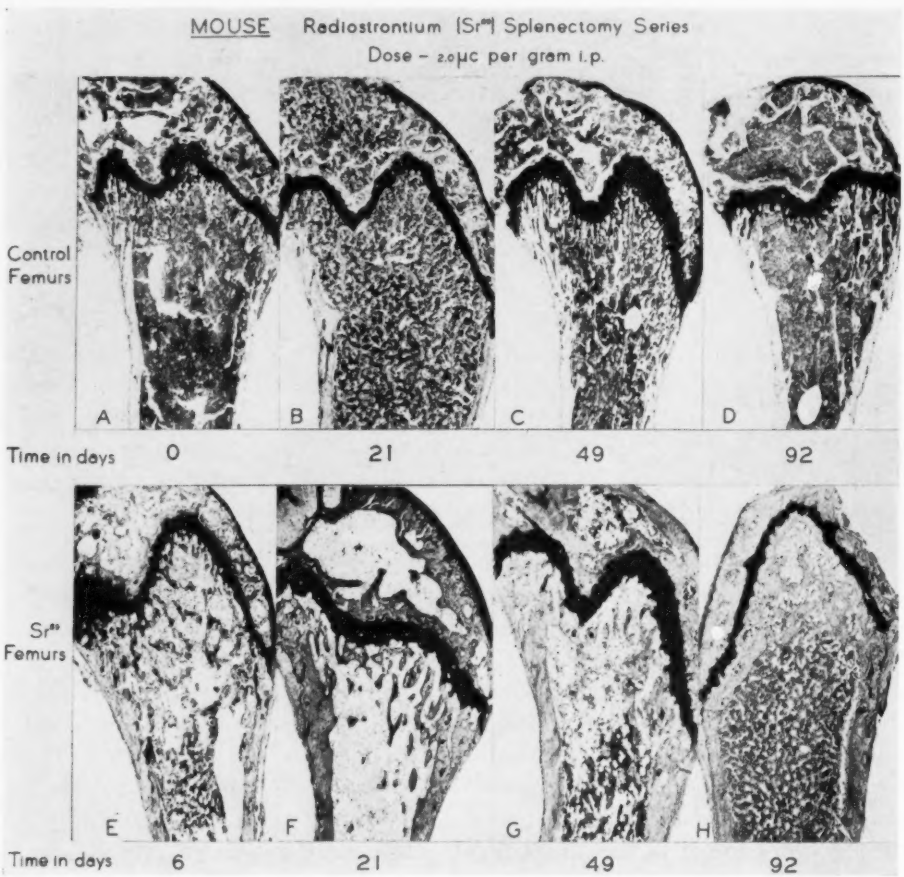


Fig. 8.—Photomicrographs of normal femoral marrow and of femurs of $Sr^{89}$-injected animals at various intervals after the $Sr^{89}$ injection. These photomicrographs illustrate the depletion of normal hematopoietic cells in the epiphyseal and metaphyseal regions with partial sparing in the diaphysis of some animals. (Hematoxylin-eosin-azure II, ×15.)

metaphyseal marrow space of the femur was present three days after injection. The general cellularity of the diaphysis of the femur as well as in the vertebral marrow space was moderately decreased. In the epiphyseal and metaphyseal regions of the femur only a few scattered heterophils, plasma cells, and large lymphocytes remained at this stage, whereas dilated vascular spaces and increased fat cells filled the area. Distally from the depleted metaphyseal region, the cellularity of the marrow gradually increased. The diaphyseal marrow was diffusely cellular but distinctly less so than that of

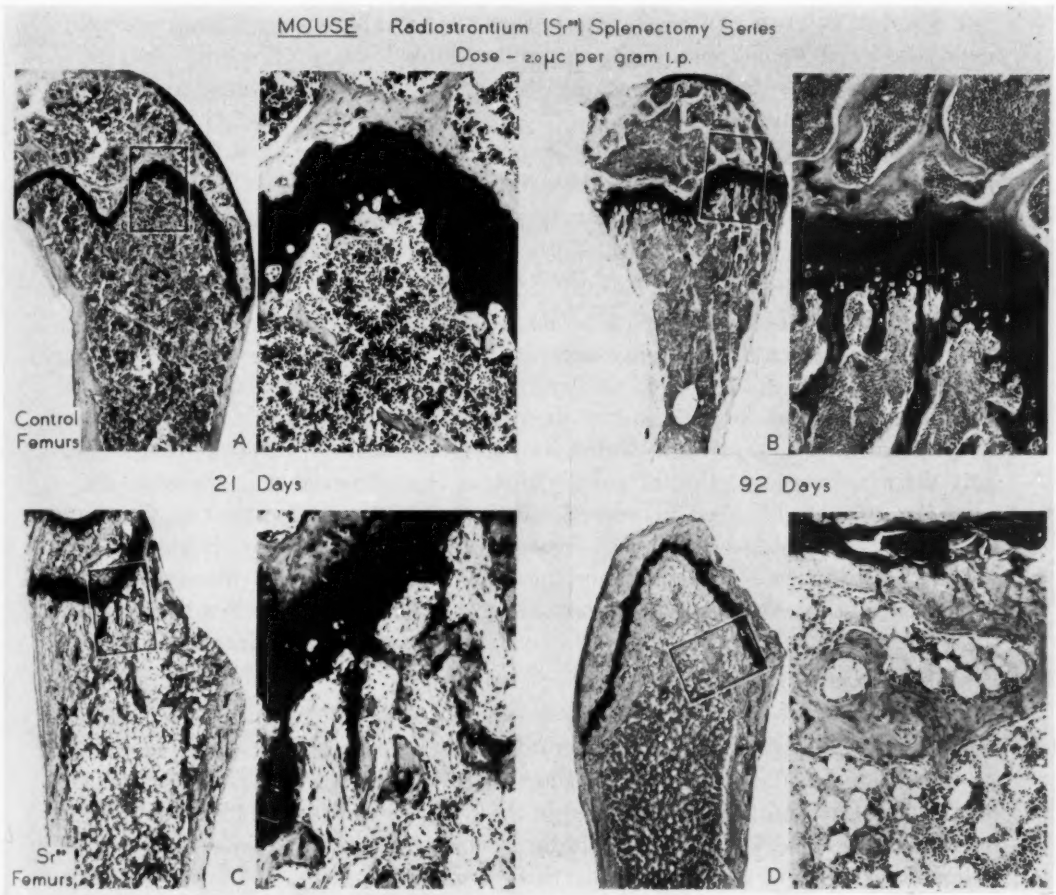


Fig. 9.—Photomicrographs of normal femoral marrow and femurs of $Sr^{89}$-injected animals with selected areas at a higher power to illustrate the loss of normal cellularity and replacement by fibrous tissue (*C*) twenty-one days after $Sr^{89}$ injection and the residual cellular depletion in the metaphysis ninety-two days after $Sr^{89}$ injection. (Hematoxylin-eosin-azure II; low power ×15, high power ×70.)

normal control mice. The vascular spaces in this part of the marrow at three days were conspicuously dilated; megakaryocytes were fewer in number; and erythrocytopoiesis was definitely decreased. The remaining cells were thus largely composed of the precursors of the granulocytic series.

The vertebral marrow was comparable with that of the femoral diaphysis at this stage. At six days the findings were essentially the same as those for the three-day stage (Fig. 8, *E*). The degree of marrow depletion varied considerably from animal to animal at various time intervals after injection. In Fig. 8, *F* the femur of a mouse sacrificed twenty-one days after injection is shown. In this specimen only fat and dilated vascular spaces filled with erythrocytes remained. In two other mice sacrificed at this same interval the epiphyseal and metaphyseal regions of the femurs were largely depleted of cells; the diaphyseal portions contained about as many hematopoietic cells as the controls. In still another mouse sacrificed at this interval the vertebral

and femoral marrow contained small areas of normal-appearing hematopoietic cells scattered about among the fat cells, dilated vascular spaces, and gelatinous areas; whereas the metaphyseal portion was more or less completely replaced by fibrous tissue (Fig. 9, *C*). At later stages, i.e., 49, 71, 92, and 119 days after $Sr^{89}$ injection, the depletion of cells in the metaphyseal and epiphyseal portions of the femoral marrow remained conspicuous (Figs. 8, *G* and 9, *D*). The shaft or diaphyseal portion of the femur and the vertebral marrow approached more normal hematopoietic activity. Likewise, in these stages the amount of fat and the dilation of the vascular spaces became less conspicuous.

*(e) Observations on Bone Marrow After Second $Sr^{89}$ Injection:* Selected animals were given a second injection of $Sr^{89}$ 119 days after the first injection. At this time (Figs. 1 and 2) no anemia existed in either group which had originally received $Sr^{89}$. Residual partial cellular depletion or fibrosis in the epiphyseal and metaphyseal region of the femur still persisted at this stage, but the diaphyseal portion of the femur and the vertebral marrow were only slightly less cellular than in normal mice. Sacrifices of mice that were given a second $Sr^{89}$ injection were made fifteen, twenty-four, and seventy-seven days after the injection. The effect on the marrow of these mice was essentially the same as that observed in comparable stages after the first injection except that the number of pigment-filled macrophages was slightly increased in the reinjected animals.

*(f) The Effect of $Sr^{89}$ on Other Tissues or Organs:* The tissues or organs, not hitherto discussed, which were studied were skin, lung, adrenal, small intestine, kidney, and liver. No cytologic evidence of destructive effect which could be attributed to $Sr^{89}$ was noted in these organs or tissues. In an occasional animal, which had been given a single injection of $Sr^{89}$ or reinjection with a comparable dose of $Sr^{89}$, ectopic erythrocytopoiesis was found in the liver and adrenal. In each instance, however, this was minimal.

## DISCUSSION

In spite of the extensive cellular depletion that develops in the bone marrow of mice injected with $Sr^{89}$ in a dose of 2 microcuries per gram of body weight, no anemia develops. Intense ectopic erythrocytopoiesis occurs with such rapidity in the spleens of these mice that the steady state of the circulating erythrocytes remains essentially normal. Splenectomized mice, on the other hand, develop an anemia after the injection of this dose of $Sr^{89}$ because of the fact that compensatory ectopic erythrocytopoiesis in the spleen is not possible and its development in other potential sites is not adequate. Increased ectopic megakaryocytopoiesis in the spleens of $Sr^{89}$-injected animals occurred as rapidly as ectopic erythrocytopoiesis, but since platelet determinations on the peripheral blood were not performed, no statement can be made with reference to whether or not this compensation was sufficient to prevent platelet reduction. In experiments suggested by the results related in this communication Jacobson and associates[10] showed that lead protection of the surgically mobilized spleens of mice during the administration of 600 r x-radia-

tion to the balance of the body obviated the development of anemia and significantly reduced the severity and the duration of the leucopenia and thrombocytopenia which regularly follow the delivery of this dose to the whole animal. Histologic studies showed that this phenomenon was due to the rapid development of ectopic blood formation in the lead-protected spleens.

An interesting problem that has been posed by this experiment relates to the fact that lymphocytopoiesis was markedly reduced in the spleens of $Sr^{89}$-injected animals, whereas the lymphatic tissue in the lymph nodes from the root of the mesentery, the thymus, and in Peyer's patches of the small intestine was essentially unaltered at any stage studied. The amount of $Sr^{89}$ found in the spleen of injected animals was minimal twenty-four hours after injection and practically none was found three days after injection. It seems unlikely, therefore, that the reduction in lymphatic tissue in the spleen extending for more than ninety days after $Sr^{89}$ injection could be attributed to the transient presence of $Sr^{89}$ in the spleen. Neither can one explain this reduction in splenic lymphocytopoiesis adequately on the basis of radiation originating from $Sr^{89}$ deposited in bone since one would expect other lymphatic tissue (lymph nodes, etc.) to be affected in a comparable manner. It would be difficult to explain the reduction in lymphatic tissue in the spleen on an indirect basis for the same reason. Although differences in the radiosensitivity of lymphatic tissue in various sites throughout the body of the mouse may exist, it seems unlikely that such an explanation is warranted here. The most plausible explanation for the decrease in splenic lymphatic tissue is that it may be related to a nutritional competition between the lymphatic tissue and ectopic hematopoiesis. The compensatory granulocytopoiesis in the spleen of $Sr^{89}$-injected mice reached a maximum at a time approximately comparable with the time at which the heterophils in the peripheral blood returned to normal values (forty-five to seventy days).

The delay in the appearance of ectopic granulocytopoiesis in the spleens of these animals suggests that this process was suppressed either primarily or secondarily, that the stimulus for an intensification of this process was less marked, or that even with a maximum stimulus the spleen had only a capacity for a gradual intensification of ectopic granulocytopoiesis.

The fact that anemia recurred in the splenectomized mice after the administration of a second injection of $Sr^{89}$ at 119 days indicates fairly conclusively that ectopic erythrocytopoiesis in sites other than the spleen is wholly inadequate to cope with the destruction and suppression of erythrocytopoiesis which occurs in the bone marrow. The eventual recovery of splenectomized mice from the anemia induced by the first $Sr^{89}$ injection must therefore have been largely a function of the recovery of erythrocytopoiesis in the marrow spaces.

The fact that histologic evidence of destruction in the bone marrow and spleen other than cellular depletion and the late appearance of pigment-filled macrophages was not seen in $Sr^{98}$-injected animals should perhaps be com-

mented upon. For example, as early as three days after $Sr^{89}$ injection, partially to essentially complete depletion of hematopoietic cells in the bone marrow was seen. Only dilated vascular spaces, fat cells, a few debris-filled macrophages and occasional plasma cells, lymphocyte-like cells, etc., remained in the bone marrow of some of the animals sacrificed at this stage. It is likely that debris-laden macrophages, pyknotic nuclei, etc., would have been more in evidence had specimens been taken earlier than three days. Likewise, in the white pulp of the spleens of $Sr^{89}$-injected animals, active phagocytosis was minimal even though the general cellularity was markedly decreased in three days.

## SUMMARY AND CONCLUSIONS

Radiostrontium ($Sr^{89}$), a $\beta$-ray emitter with a half-life of fifty-five days, is largely deposited in the skeleton when administered to the experimental animal. Although deposition in bone is general, the greatest concentration of this isotope invariably occurs in areas of active bone growth.

A persistent leucopenia but no anemia of significance was produced in the peripheral blood of young mice given a single intraperitoneal injection of this isotope as the chloride in a dose of 2.0 microcuries per gram. The same dose produced a leucopenia and an anemia in splenectomized mice. Recovery from the anemia was essentially complete by 119 days after the $Sr^{89}$ injection. Hematopoiesis was markedly reduced in the bone marrow of all radiostrontium-treated animals within three days after injection. The epiphyseal and metaphyseal regions of the long bones were largely depleted of cells, or, in some instances, the latter regions were completely replaced by fibrous tissue. Recovery of hematopoiesis in the bone marrow of the femur was essentially complete by 119 days except that some cellular depletion still existed in the metaphysis at this stage.

Ectopic erythrocytopoieses and megakaryocytopoiesis were greatly increased in the spleen three days after $Sr^{89}$ injection as was true of all other intervals studied through 119 days. Splenic lymphocytopoiesis, on the other hand, was reduced in three days and recovery was not complete by 119 days. Lymphocytopoiesis was essentially unaffected in other lymphatic tissues (lymph nodes, thymus, etc.). Ectopic granulocytopoiesis increased more slowly in the spleens of $Sr^{89}$-injected animals, reaching a maximum at circa sixty days. Ectopic granulocytopoiesis, megakaryocytopoiesis, and erythrocytopoiesis were not remarkable in other tissues of the animals which received $Sr^{89}$.

The rapid development and the persistence of ectopic erythrocytopoiesis in the spleen prevent anemia in mice given $Sr^{89}$ intraperitoneally in a dose of 2.0 microcuries per gram.

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# JAPANESE B ENCEPHALITIS: REPORT OF FIVE CASES

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## INTRODUCTION

THE epidemic of Japanese B encephalitis in Japan in the summer of 1948 was of significant interest because of the opportunity it afforded for direct observations on this exotic disease by physicians of the occupation forces. From a panoramic public health viewpoint, it was essentially of minor import as the actual percentage of the population afflicted was exceedingly small. In view of the rapidly expanding geographical confines of the disease, it may present a diagnostic and therapeutic problem to clinicians in the United States in the near future.

## EPIDEMIOLOGICAL AND ETIOLOGICAL ASPECTS

The purpose of this article is to point out the clinical observations on a small series of five serologically proved cases observed at the Navy Dispensary, Yokosuka, Japan. Nevertheless, it does not seem amiss to discuss some of the features of the epidemiological and etiological aspects.

The geographical distribution of the disease is rather widespread, cases having been reported from Okinawa and the near-by islands, Korea, China, Guam (epidemic of 1947), Siberia, the Japanese islands, and possibly many other countries of the Far East.[1, 2, 3]

*Vectors.*—In our present state of knowledge, it is believed that the encephalitic virus is conveyed by a mosquito. Inada,[4] a Japanese investigator, and two Russian workers, Petrischeva and Shublada,[5] have reported the isolation of the virus from *Culex tritaeniorhynchus* and *Culex pipiens* var. *pallens*. Animal reservoirs of infection have been discovered in horses, pigs, goats, cows, and possibly sparrows, but not in chickens.[1, 2] It is interesting to note, as demonstrated in studies in Japan, that outbreaks among domestic animals may occur coincident with no or few human clinical cases.[6] Presumably epidemic outbreaks may occur only when the factors of mosquitoes, animal reservoirs of infection, and human susceptibles exist concomitantly. The disease has presented a fluctuating course in Japan, reaching epidemic or minor epidemic proportions every few years, a feature probably related to the accumulation of susceptible persons. In July, 1924, there were 6,125 cases with a mortality rate of 62 per cent. In 1935, there was an outbreak of 5,370 cases.[7] One interesting aspect of the epidemic under discussion is that the conditions of temperature and rainfall were exceedingly favorable for the propagation of mosquitoes prior to the outbreak of the disease.

*Incidence in Man.*—Apparently, from evidence accumulated from many sources, the clinically recognizable cases represent only a relatively small

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The opinions or assertions contained herein are the private ones of the writer and are not to be construed as official or reflecting the view of the Navy Department or the naval service at large.

Received for publication, Aug. 12, 1949.

proportion of the total, the majority smoldering under the blanket of subclinical or very mild clinical infection. In 1936, one year after a large epidemic, 83 per cent of 116 sera tested in Tokyo revealed virus neutralizing antibodies.[8] In 1937, 0.8 per cent of 525 human sera obtained from Hokkaido, Japan, demonstrated these antibodies.[8] Hokkaido is not an epidemic nor an endemic area of the disease. In 1941, 83 per cent of 104 sera from Middle China possessed neutralizing ability against the Japanese B virus,[8] and, in 1945, 90 per cent of the natives of Okinawa, similarly, had neutralizing antibodies.[1] In 1946, a similar phenomenon was found in the sera of 85 per cent of thirteen Chinese residents of Shanghai and 89 per cent of nineteen Chinese residents of Tientsin.[3]

Sabin[1] reported a mortality rate for the 1945 outbreak on Okinawa of 28.6 per cent, and on the near-by islands of Heanya and Hamahika, 30.6 per cent. Among American military forces on Okinawa in 1945, there were two deaths occurring in a series of twelve severe cases. Figures compiled by the Public Health and Welfare Section, Supreme Commander for Allied Powers, revealed that in the first forty-one weeks of 1948 there were approximately 8,000 cases of Japanese B encephalitis throughout the empire, and 2,374 deaths were reported. The approximate mortality rate from the disease was 25.7 per cent for the Tokyo area and 35.7 per cent for all Japan.[7] In the summer of 1948, there were three fatalities in the twenty-nine proved cases of Japanese B encephalitis in occupation personnel in Japan. Because of the paucity of serologically proved cases occurring among occupation personnel in Japan, a study of the features of the disease as manifested in the five cases forming the subject of this paper seemed all the more desirable.

Although superficially the number of deaths in 1948 attributable to the encephalitic virus seems fairly large, it shrinks considerably when viewed relative to the 78 millions inhabiting the Japanese islands. These fatalities fade almost into oblivion when one contemplates the staggering total of approximately 147,000 deaths in Japan in 1947 directly attributable to tuberculosis. According to several attending Japanese physicians, approximately 2 per cent of the patients exhibit sequelae. These include clonic muscular movements, decerebrate rigidity, and twitchings of muscle groups.

The figures for age incidence in the Tokyo area in 1948[7] reveal a high rate for the younger age group, i.e. through 1 to 20 years of age. The highest occurred in the 6 to 10 years group, the rate being 117.3 per 100,000. The lowest incidence occurred in the 31 to 70 years group, the rate being 9.1 per 100,000. In the early years, male patients have the highest incidence of the disease, but this tendency decreases after the age of 20 until there is a female preponderance in the 51 to 60 years group. In all ages, the mortality among female patients is higher than that among male patients. The over-all case fatality rate in Tokyo during the 1948 epidemic was 19.9 per cent for males and 33.3 per cent for females. These figures were derived from 2,067 cases.

Two cases, occurring in nonvaccinated Naval personnel, are of interest in that they assist in elucidating the time requisite for the incubation period of the

disease. Two firemen from a Naval vessel stayed ashore overnight in Yokohama. The ship remained in Yokohama for two days, sailing Aug. 1, 1948. Many mosquitoes were observed aboard ship. On August 11 one man reported to the ship's sick bay with a complaint of headache of four hours' duration. He seemed drowsy and had a fever. The patient was transferred to a British hospital at Ceylon thirty hours later and died twenty-four hours after transfer. Unfortunately, no autopsy was performed. On August 21 the other fireman developed a headache and fever and became disoriented. A spinal fluid examination performed at a hospital at Bahrein Gulf showed a slight pleocytosis and an increase in protein. The patient's clinical course was similar to that of cases of Japanese B encephalitis occurring in Japan. Following complete recovery, the man returned to Japan. His complement-fixing titer one month after the original infection was 1:16, and the neutralization index was 63,000.

*Status of Vaccination.*—The status of vaccine has not been established at this time. No conclusions relative to the efficacy of vaccination could be drawn from the epidemic on Okinawa. Sabin[9] has shown a rise in neutralization and complement-fixing antibodies in young people inoculated with mouse brain vaccine, two 1 c.c. doses being given six days apart and the last dose of 1 c.c. one month later. Very elderly persons who had no previous serologic neutralizing antibodies exhibited no response. Following the administration of a potent vaccine, many persons develop a rise in neutralizing antibodies.[7] In American adults, no rise in complement-fixing antibodies occurs as a result of vaccination alone, although a person previously infected with the live virus may develop complement-fixing antibodies after vaccination.[7] The vaccine used at present is a chick embryo vaccine, and its efficacy is under intense investigation.

Twelve patients of the twenty-nine cases occurring among occupational personnel in Japan had received complete immunization. No deaths occurred among this group. Three patients had received complete immunization except for one dose. No deaths occurred among these. Six patients had failed to obtain complete vaccination by two or more doses. There was one death among them. Two deaths occurred among eight completely nonimmunized patients.[7]

*Symptoms.*—The clinical symptoms of the disease as previously reported by Sabin[1, 2] include marked lethargy, confusion, high fever, nuchal rigidity, leucocytosis, and spinal fluid pleocytosis. The onset is abrupt, with sudden headache and fever. Sabin, in his Okinawa series[1] (ten serologically proved), observed convulsions in only two cases, both of them fatal. He stressed the duration of the fever, seven to eleven days, and the constant bradycardia present. Cells in the spinal fluid were predominantly mononuclear, whereas a polymorphonuclear leucocytosis was found in the peripheral blood smear. One patient of his series exhibited interesting sequelae, i.e., hyperirritability, masklike facies, somnolence, and a propensity for urinating on the floor. In the Korean outbreak, two instances of bladder paralysis were recorded. One of these patients died. In the other, bladder function became normal upon convalescence.

*Serologic Studies.*—Serologic studies upon the Okinawa cases revealed interesting data. Serum neutralizing antibodies were questionably or certainly positive on the fourth or fifth day of the disease. Low titers of complement-fixing antibodies were found as early as two to eight days after the onset. However, these complement-fixing antibodies may not appear until the fifth week. No discrepancies were noted between the results of the neutralization and complement-fixing tests. For several technical reasons, Sabin feels that the complement-fixing test is more practical. It is interesting that many cases, initially suspected of being mild or abortive cases, in his Okinawa series gave no serologic evidence of Japanese B encephalitis.

*Pathology.*—Gross pathology reveals a diffuse congestion of the brain, sometimes with softening of the cervical cord.[10] There is diffuse involvement of the leptomeninges and the gray matter. Perivenous lymphocytes are predominant in the arachnoid meshes. The meningeal lesions are widespread over the surface of the brain, being minimal over the midbrain, pons, medulla, cerebellum, and spinal cord. Many nodules composed of mononuclear cells, clustered about degenerating ganglion cells or blood vessels, occur in the gray matter. The cortex, thalamus, substantia nigra, nuclei of the fourth ventricle, cerebellar cortex, and the anterior horns of the spinal cord are involved. Adrenal "tubular degeneration" may occur.

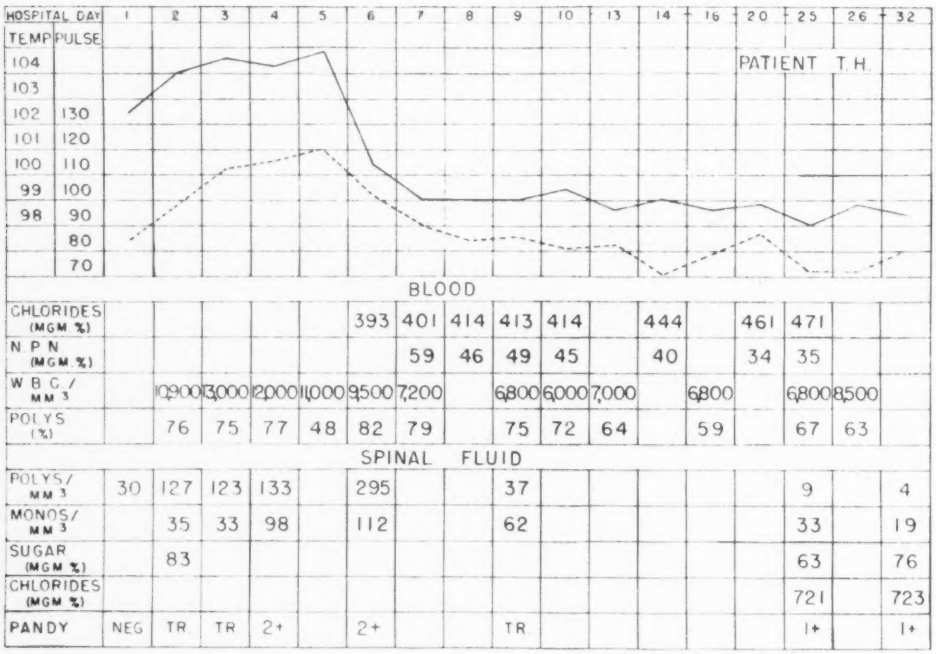
## CASE REPORTS

CASE 1.—Patient T. H., a 23-year-old white man, entered the Navy Dispensary on Aug. 8, 1948, complaining of a headache beginning the previous day. This headache began suddenly, progressively became worse, and was accompanied by malaise and chills. He had completed the course of immunization against Japanese B encephalitis approximately two months before the present illness.

*Physical Examination and Laboratory Data:* T., 102.4° F.; P., 84; R., 22; B.P., 116/78 mm. Hg. The patient appeared flushed, dehydrated, and acutely ill. There was definite nuchal rigidity. The reflexes were physiologic, and Kernig's sign was negative. The spinal fluid showed 30 polymorphonuclear cells, and the Pandy reaction was negative. Blood leucocyte count on the second hospital day was 10,900 with 76 per cent polymorphonuclear cells. Admission urinalysis was negative. Initial chest films, as well as all subsequent chest films, were negative.

*Course:* Intravenous fluids, oral sulfadiazine, and intramuscular penicillin were instituted. On the second hospital day the fever rose to 104° F. There were 162 white cells in the spinal fluid, of which 127 were polymorphonuclear. During the next two days the temperature climbed to 104.6° F., and there was no essential change in the neurological picture. The patient was relatively alert. On the fourth hospital day the spinal fluid contained 133 polymorphonuclear and 98 mononuclear cells, and the Pandy reaction at this time was two plus. The patient became completely disoriented on the fifth hospital day. He did not respond to questioning, had to be restrained in bed, and was maniacal. Paraldehyde was necessary for sedation. There were definite neurological changes. The arms developed a lead-pipe type of rigidity, and there were marked gross tremors of the upper extremities and tongue with twitchings of the mouth. On the sixth hospital day, the temperature suddenly dropped and remained normal on the seventh day. The patient became more rational. A lumbar puncture at this time revealed approximately 400 cells in the spinal fluid, about three-fourths of them being polymorphonuclear, and a two plus Pandy reaction. Improvement after the seventh hospital day was rapid, and the temperature remained essentially

normal. During the subsequent thirteen days, the mental cloudiness, muscle twitching, and muscle tremors disappeared as well as all traces of nuchal rigidity. However, the patient's voice, which had become quite husky, remained so for two months. This has cleared completely at the time of the present writing, i.e., approximately five months from the onset of the illness. When the patient became ambulatory again, he complained bitterly of dizziness when walking, but this complaint rapidly disappeared.



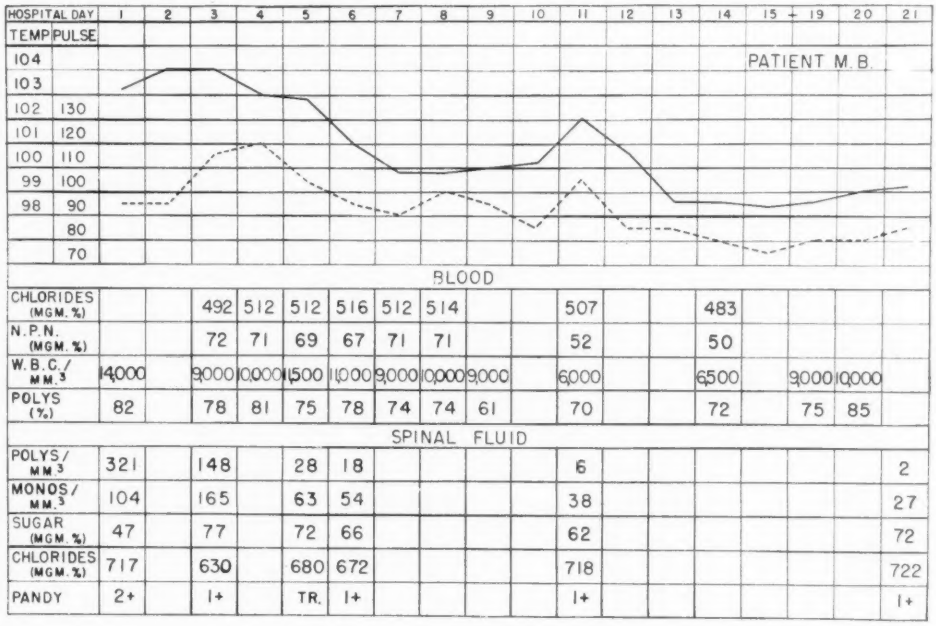

| HOSPITAL DAY | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 13 | 14 | 16 | 20 | 25 | 26 | 32 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| CHLORIDES (MGM %) | | | | | | 393 | 401 | 414 | 413 | 414 | | 444 | | 461 | 471 | | |
| N P N (MGM %) | | | | | | | 59 | 46 | 49 | 45 | | 40 | | 34 | 35 | | |
| W B C / MM 3 | | 10,900 | 13,000 | 12,000 | 11,000 | 9,500 | 7,200 | | 6,800 | 6,000 | 7,000 | | 6,800 | | 6,800 | 8,500 | |
| POLYS (%) | | 76 | 75 | 77 | 48 | 82 | 79 | | 75 | 72 | 64 | | 59 | | 67 | 63 | |
| POLYS/ MM 3 | 30 | 127 | 123 | 133 | | 295 | | | 37 | | | | | | 9 | | 4 |
| MONOS/ MM 3 | | 35 | 33 | 98 | | 112 | | | 62 | | | | | | 33 | | 19 |
| SUGAR (MGM %) | | 83 | | | | | | | | | | | | | 63 | | 76 |
| CHLORIDES (MGM %) | | | | | | | | | | | | | | | 721 | | 723 |
| PANDY | NEG | TR | TR | 2+ | | 2+ | | | TR | | | | | | 1+ | | 1+ |

Fig. 1.

CASE 2.—Patient M. B., a 37-year-old white man, entered the Navy Dispensary on Aug. 28, 1948. The patient stated that the evening before he had experienced sudden severe headache and chills. He remembered starting to the Dispensary the next morning, but affirmed later that he did not have any recollection of his arrival nor of the immediately subsequent events. There had been no immunization against Japanese B encephalitis.

*Physical Examination and Laboratory Data*: T., 103.2° F.; P., 96; R., 22; B.P., 142/78 mm. Hg. Within a few moments after admission to the medical ward, the patient experienced a severe clonic convulsion with attendant cyanosis. The convulsion lasted approximately five minutes, and upon its cessation the patient was unruly and completely irrational, necessitating restraint by several men. The patient was disoriented and could make no intelligent reply to questioning. The administration of intravenous Sodium Amytal was necessary. There was flushing of the face and extreme dehydration. A foul discharge, from which pneumococci were subsequently cultured, drained from a right otitis externa. The mucous membranes of the throat were injected, and the tongue had been traumatized during the convulsion. A Grade II apical systolic murmur was present. There was marked nuchal rigidity. Kernig's sign was positive. All reflexes were hypoactive, the abdominal and ankle reflexes were absent, and the Babinski was positive bilaterally. The spinal fluid contained 425 cells, of which 321 were polymorphonuclear cells. The Pandy reaction was two plus. The initial blood leucocyte count was approximately 14,000 with 82 per cent neutrophils. The urine contained a trace of protein. The chest film was negative.

*Course*: Therapy, in the form of oral sulfadiazine, intramuscular penicillin, intravenous fluids, and intranasal oxygen, was instituted. On the second hospital day, the patient developed a lead-pipe rigidity of the arms and gross tremors of the lips and upper extremities. Similar but less marked rigidity of the lower extremities was also evident. This clinical picture persisted for six days. On the seventh day the temperature dropped to 99.8° F. The gross tremors of the lips, tongue, and arms were still present. The ankle jerks




| HOSPITAL DAY | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 + 19 | 20 | 21 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| TEMP / PULSE | | | | | | | | | | | | | | | | | |
| 104 | | | | | | | | | | | | | | | | | |
| 103 | | | | | | | | | | | | | | | | | |
| 102 / 130 | | | | | | | | | | | | | | | | | |
| 101 / 120 | | | | | | | | | | | | | | | | | |
| 100 / 110 | | | | | | | | | | | | | | | | | |
| 99 / 100 | | | | | | | | | | | | | | | | | |
| 98 / 90 | | | | | | | | | | | | | | | | | |
| 80 | | | | | | | | | | | | | | | | | |
| 70 | | | | | | | | | | | | | | | | | |
| BLOOD | | | | | | | | | | | | | | | | | |
| CHLORIDES (MGM. %) | | | 492 | 512 | 512 | 516 | 512 | 514 | | | 507 | | | 483 | | | |
| N.P.N. (MGM. %) | | | 72 | 71 | 69 | 67 | 71 | 71 | | | 52 | | | 50 | | | |
| W.B.C./MM.$^3$ | 14,000 | | 9,000 | 10,000 | 11,500 | 11,000 | 9,000 | 10,000 | 9,000 | | 6,000 | | | 6,500 | 9,000 | 10,000 | |
| POLYS (%) | 82 | | 78 | 81 | 75 | 78 | 74 | 74 | 61 | | 70 | | | 72 | 75 | 85 | |
| SPINAL FLUID | | | | | | | | | | | | | | | | | |
| POLYS/MM.$^3$ | 321 | | 148 | | 28 | 18 | | | | | 6 | | | | | | 2 |
| MONOS/MM.$^3$ | 104 | | 165 | | 63 | 54 | | | | | 38 | | | | | | 27 |
| SUGAR (MGM. %) | 47 | | 77 | | 72 | 66 | | | | | 62 | | | | | | 72 |
| CHLORIDES (MGM. %) | 717 | | 630 | | 680 | 672 | | | | | 718 | | | | | | 722 |
| PANDY | 2+ | | 1+ | | TR. | 1+ | | | | | 1+ | | | | | | 1+ |

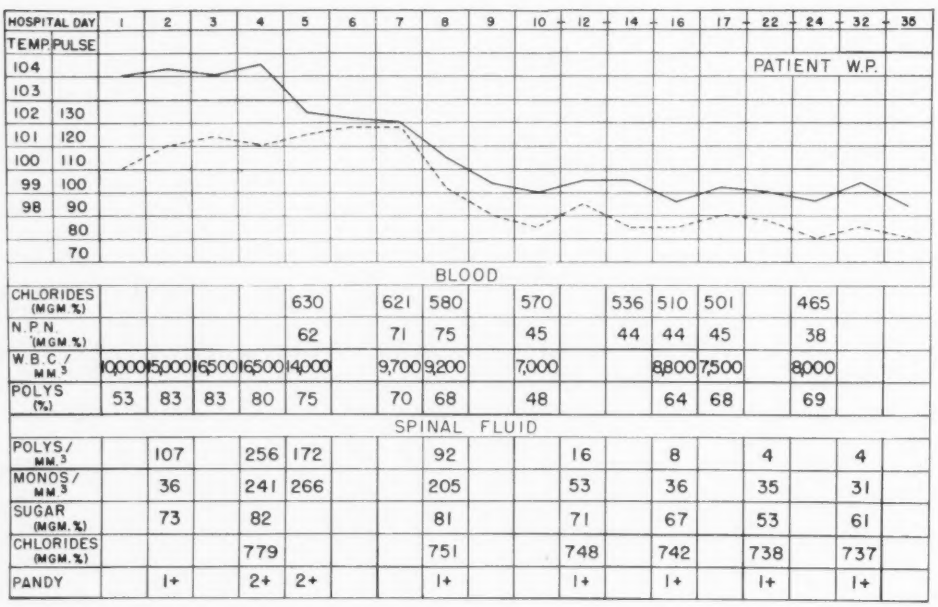
Fig. 2.

were absent, the Babinski was negative, and all other reflexes were hypoactive. Transient weakness of the external ocular rectus muscles appeared. It was necessary to discontinue oxygen for approximately thirty minutes because of a mechanical defect in the apparatus, and at the end of that time it was noted that the slightest stimulus could precipitate violent movements bordering on a convulsion. Following resumption of oxygen inhalation for a few minutes, the patient became much more responsive and much less hyperirritable. After the eighth hospital day he became much clearer mentally and rapidly regained his normal sensorium. Likewise, the muscle tremors cleared. Neurological abnormalities disappeared, and no physical sequelae of the encephalitis remained. His convalescence was complicated by a spondylitis.

CASE 3.—Patient W. P., a 24-year-old white man, entered the Navy Dispensary on Aug. 19, 1948, complaining of headache, fever, malaise, and nuchal rigidity of two days' duration. He had been immunized against Japanese B encephalitis in 1946 and had received a booster dose of 1 c.c. in 1947 and in June, 1948.

*Physical Examination and Laboratory Data*: T., 104° F.; P., 110; R., 24; B.P., 122/74 mm. Hg. The patient was acutely ill and markedly dehydrated. The skin was red and flushed. There was slight stiffness of the neck and transient weakness of the external rectus muscles of both eyes. All reflexes were normal, and the Babinski signs were normal. Initial spinal fluid studies revealed 143 white cells, 107 of them being polymorphonuclear, and a one plus Pandy reaction. The blood leucocyte count was 10,000 with 53 per cent polymorphonuclear cells. The urinalysis was negative. Initial and subsequent chest films were normal.

*Course*: Intravenous fluids, oral sulfadiazine, and intramuscular penicillin were administered. On the second hospital day the fever was 104.2° F., and there was no significant change in the clinical picture. On the third hospital day the patient complained of difficulty in breathing and became lethargic. Nuchal rigidity was more marked by this time, but there were no reflex changes. He became completely unresponsive the next day, at which time the temperature was 104.5° F. A lumbar puncture on this date revealed 256 polymorphonuclear cells and 241 mononuclear cells in the spinal fluid. The Pandy reaction at this time was two plus. There was no cyanosis nor evidence of cardiovascular impairment.

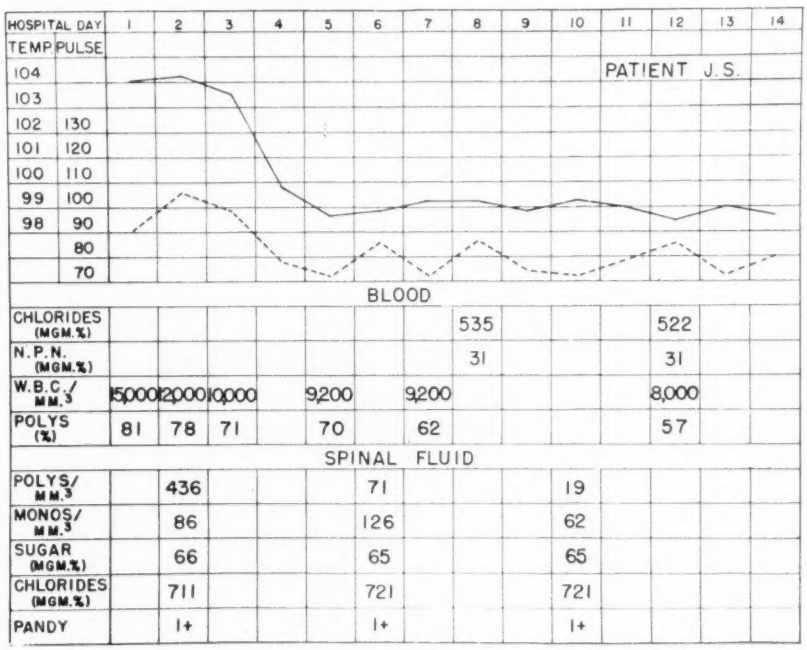



| HOSPITAL DAY | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 12 | 14 | 16 | 17 | 22 | 24 | 32 | 38 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| TEMP. PULSE | | | | | | | | | | | | | | | | | | |
| 104 | | | | | | | | | | | | | | | | | | |
| 103 | | | | | | | | | | | | | | | | | | |
| 102 | 130 | | | | | | | | | | | | | | | | | |
| 101 | 120 | | | | | | | | | | | | | | | | | |
| 100 | 110 | | | | | | | | | | | | | | | | | |
| 99 | 100 | | | | | | | | | | | | | | | | | |
| 98 | 90 | | | | | | | | | | | | | | | | | |
| | 80 | | | | | | | | | | | | | | | | | |
| | 70 | | | | | | | | | | | | | | | | | |
| BLOOD | | | | | | | | | | | | | | | | | | |
| CHLORIDES (MGM. %) | | | | | 630 | | 621 | 580 | | 570 | | 536 | 510 | 501 | | 465 | | |
| N.P.N. (MGM. %) | | | | | 62 | | 71 | 75 | | 45 | | 44 | 44 | 45 | | 38 | | |
| W.B.C. / MM.$^3$ | 10,000 | 15,000 | 16,500 | 16,500 | 14,000 | | 9,700 | 9,200 | | 7,000 | | | 8,800 | 7,500 | | 8,000 | | |
| POLYS (%) | 53 | 83 | 83 | 80 | 75 | | 70 | 68 | | 48 | | | 64 | 68 | | 69 | | |
| SPINAL FLUID | | | | | | | | | | | | | | | | | | |
| POLYS / MM.$^3$ | | 107 | | 256 | 172 | | | 92 | | | 16 | | 8 | | 4 | | 4 | |
| MONOS / MM.$^3$ | | 36 | | 241 | 266 | | | 205 | | | 53 | | 36 | | 35 | | 31 | |
| SUGAR (MGM. %) | | 73 | | 82 | | | | 81 | | | 71 | | 67 | | 53 | | 61 | |
| CHLORIDES (MGM. %) | | | | 779 | | | | 751 | | | 748 | | 742 | | 738 | | 737 | |
| PANDY | | 1+ | | 2+ | 2+ | | | 1+ | | | 1+ | | 1+ | | 1+ | | 1+ | |

Fig. 3.

On the sixth hospital day the pulse rose to 128 and the respiratory rate accelerated to 30. There was marked tremor of the arms, lips, and tongue. Cheyne-Stokes respirations began and intranasal oxygen was administered. Later in the day the respiratory rate diminished to 22. At this time the respiratory movements were irregular but not Cheyne-Stokes in character. Knee jerks were hypoactive and the abdominal reflex was absent. Nuchal rigidity was definite, and the typical lead-pipe rigidity of the upper extremities was obvious. On the eighth hospital day, lumbar puncture revealed 92 polymorphonuclear and 205 mononuclear cells in the spinal fluid and a one plus Pandy reaction. On the evening of the same day, the patient experienced multiple convulsions, necessitating intravenous Sodium Amytal for their control. Cyanosis was demonstrable only immediately after the convulsions. Cheyne-Stokes respirations reappeared, associated with long periods of apnea. Pulmonary edema appeared suddenly, and the condition of the patient appeared grave. However, the pulmonary edema rapidly subsided after a hypodermic injection of morphine. Marked improvement occurred after this episode, and two days later the patient could respond to questioning. Twitching of the mouth, tremors of the upper extremities, and grimacing on minimal stimuli remained. The knee jerks and right ankle jerk could not be elicited. The temperature was now 99° F. By the thirteenth hospital day the patient was cheerful and alert. Both ankle jerks were now present, but the knee jerks were still absent. On this day the patient developed a seventh nerve palsy of the peripheral type on the right side of the face. This nerve weakness disappeared during the course of the next two weeks. Gross muscle tremors and twitches likewise receded. On the sixteenth hospital day the patient complained of

complete anesthesia of the dorsal and palmar surfaces of the index and middle fingers of the left hand. Neurological examination revealed no sensation in these areas. The sensory disturbance partially cleared in a few days, but marked weakness of the adductor of the left thumb and flexors of the left forefinger occurred. In a two-month follow-up, it was demonstrated that sensation over the fingers was normal and that the muscular weakness had improved on physiotherapy. Atrophy of the left thenar eminence was present.




| HOSPITAL DAY | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| TEMP / PULSE | | | | | | | | | | | | | | |
| 104 | | | | | | | | | | | | | | |
| 103 | | | | | | | | | | | | | | |
| 102 / 130 | | | | | | | | | | | | | | |
| 101 / 120 | | | | | | | | | | | | | | |
| 100 / 110 | | | | | | | | | | | | | | |
| 99 / 100 | | | | | | | | | | | | | | |
| 98 / 90 | | | | | | | | | | | | | | |
| 80 | | | | | | | | | | | | | | |
| 70 | | | | | | | | | | | | | | |
| BLOOD | | | | | | | | | | | | | | |
| CHLORIDES (MGM.%) | | | | | | | | 535 | | | | 522 | | |
| N.P.N. (MGM.%) | | | | | | | | 31 | | | | 31 | | |
| W.B.C./MM.$^3$ | 15,000 | 12,000 | 10,000 | | 9,200 | | 9,200 | | | | | 8,000 | | |
| POLYS (%) | 81 | 78 | 71 | | 70 | | 62 | | | | | 57 | | |
| SPINAL FLUID | | | | | | | | | | | | | | |
| POLYS/MM.$^3$ | | 436 | | | | 71 | | | | 19 | | | | |
| MONOS/MM.$^3$ | | 86 | | | | 126 | | | | 62 | | | | |
| SUGAR (MGM.%) | | 66 | | | | 65 | | | | 65 | | | | |
| CHLORIDES (MGM.%) | | 711 | | | | 721 | | | | 721 | | | | |
| PANDY | | 1+ | | | | 1+ | | | | 1+ | | | | |

Fig. 4.

CASE 4.—Patient J. S., a 27-year-old white man, was admitted to the Navy Dispensary on Aug. 19, 1948, complaining of severe headache and vomiting. Four days previously he had noticed the sudden onset of headache, chills, and generalized malaise. During the twelve hours preceding admission he had experienced frequent emesis. His immunization against Japanese B encephalitis had been incomplete since he had received only two of the required three injections approximately two months before admission.

*Physical Examination and Laboratory Data*: T., 104° F.; P., 90; R., 22; B.P., 140/70 mm. Hg. The patient was quite dehydrated and flushed. He was, nevertheless, well oriented and alert. Moderate injection of the mucous membranes of the nose and throat was present. Nuchal rigidity was slight, though definite. The remainder of the neurological examination was negative. On the second hospital day there were 522 white cells in the spinal fluid, of which 436 were polymorphonuclear cells. The Pandy reaction was one plus. The initial blood leucocyte count was 15,000 with 81 per cent polymorphonuclears. The urinalysis was negative. Initial and subsequent chest films were negative.

*Course*: Therapy, consisting of intravenous fluids, oral sulfadiazine, and intramuscular penicillin, was started. The patient's course was uneventful, and on the second hospital day he appeared cheerful and alert. The temperature reached normal limits on the fifth hospital day, and nuchal rigidity cleared rapidly thereafter. During the mild course of the illness, no reflex changes nor clouding of the sensorium occurred. There were no tremors nor lead-pipe rigidity. On the fourteenth hospital day the patient was discharged to duty free of sequelae.

CASE 5.—Patient M. W., a 29-year-old white woman, entered the Navy Dispensary on Aug. 19, 1948, complaining of fever, malaise, and headache. These symptoms had begun suddenly three days before admission. The evening before admission she had become disoriented and irrational. There had been no immunization against Japanese B encephalitis.

*Physical Examination and Laboratory Data*: T., 105.5° F.; P., 120; R., 24; B.P., 110/80 mm. Hg. The patient was flushed and dehydrated. She was irrational and could not respond to questioning. A gross tremor of the upper extremities and twitching of the mouth

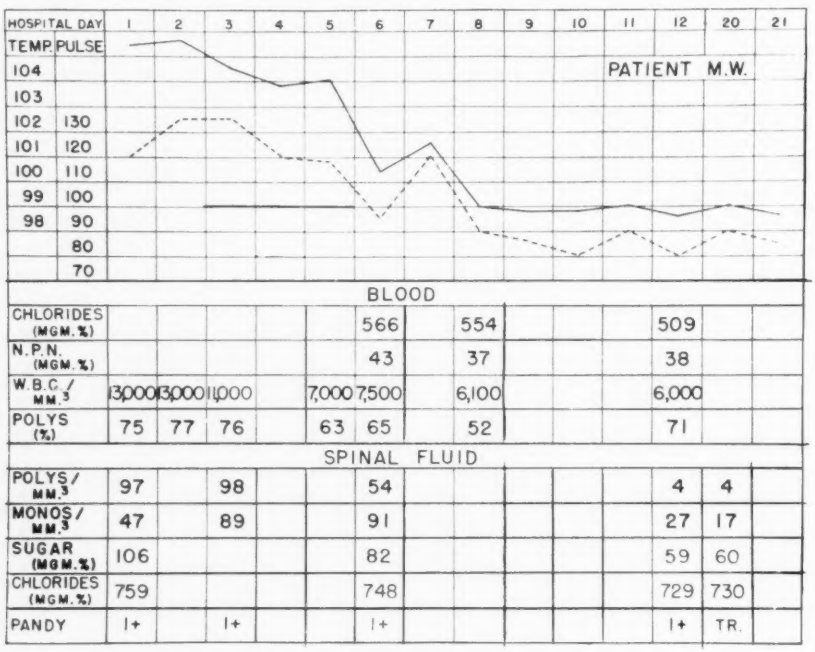



Fig. 5.

were present. Definite nuchal rigidity could be elicited. There was mild injection of the mucous membranes of the nose and throat. All reflexes were physiologic except for a questionably positive Babinski on the right. There were 97 polymorphonuclear cells and 47 mononuclear cells in the spinal fluid. The Pandy reaction was one plus. The blood leucocyte count was 13,000, 75 per cent of these cells being polymorphonuclear. The urinalysis was negative. Initial and subsequent chest films were negative.

*Course*: Intravenous fluids, oral sulfadiazine, and intramuscular penicillin were administered. During the following four days the patient remained delirious and continued to have the gross tremors described. Lead-pipe rigidity of the upper extremities also appeared. On the sixth hospital day the temperature decreased to 100.5° F. and on the seventh hospital day the patient became much clearer mentally. Thereafter improvement was rapid and all neurological abnormalities quickly disappeared. No sequelae remained.

## DISCUSSION

The diagnosis in each of the five patients was proved by a rise in complement-fixing antibodies in the serum. The results of the complement fixation tests and the neutralization indices are shown in Table I.

Six other patients, having the same type of symptoms, spinal fluid pleocytosis, and clinical picture, are omitted from this discussion because positive serologic proof was not obtained. The question arises as to whether

TABLE I. COMPLEMENT FIXATIONS AND NEUTRALIZATION INDICES

| PATIENT | HOSPITAL DAY | COMPLEMENT FIXATION* | NEUTRALIZATION INDEX |
|---|---|---|---|
| Case 1 T. H. | 20 | 1:16 | 320 |
| | 26 | 1:64 | 1,000 |
| | 35 | 1:64 | 10,000 |
| | † | 1:64 | 2,000 |
| | ‡ | 1:32 | 10,000 |
| Case 2 M. B. | 6 | Neg. | 630 |
| | 17 | 1:8 | 20,000 |
| | 21 | 1:32 | 2,000 |
| Case 3 W. P. | 2 | 1:4 | — |
| | 9 | 1:4 | 10,000 |
| | 15 | 1:32 | 10,000 |
| | 26 | 1:32 | 200,000 |
| | 35 | 1:64 | 320,000 |
| | ‡ | 1:32 | 10,000 |
| Case 4 J. S. | 2 | 1:64 | 6,300 |
| | 9 | 1:256 | 13,000 |
| | 14 | 1:32 | 13,000 |
| Case 5 M. W. | 2 | Neg. | 1,000 |
| | 9 | Neg. | 3,200 |
| | 15 | Neg. | 3,200 |
| | 21 | 1:8 | 10,000 |

*A titer over 1:4 is considered significant.
†Follow-up twelve days after discharge.
‡Follow-up five months after onset.

these were indeed cases of Japanese B encephalitis and whether it is possible that in some instances the threshold of immunologic response is not reached.

The therapeutic regimen adopted was necessarily varied to compensate for the individual needs of the patient. One of the characteristic features of the disease was the marked dehydration present initially. The nonprotein nitrogen determinations were elevated in two patients even after several days of rehydration and in a third before rehydration was accomplished. Probably the nonprotein nitrogen would have been elevated in the other two patients had this value been determined at the beginning of their hospitalizations.

Blood chloride values were generally within normal limits, and blood nonprotein nitrogen determinations were consistent with the degree of dehydration evidenced by the patient. In no patient could it be demonstrated that renal disease was a factor in the elevation of the nonprotein nitrogen. A few fasting blood sugar determinations were normal. Several blood calcium determinations in two patients experiencing convulsions were normal.

Large quantities of saline and glucose were administered intravenously until adequate oral intake could be assured. As these individuals were quite young, large quantities could be infused with relative safety. In all cases, adequate urinary flow was achieved. In no instance did edema occur except in one patient who developed pulmonary edema following repeated convulsions. This edema quickly responded to morphine.

Intravenous barbiturate sedation was essential in two men to control multiple convulsions. Paraldehyde was requisite for one man who had become aggressive and maniacal.

Sulfadiazine and penicillin were instituted in all patients because, at that time, the exact nature of the disease had not been ascertained. It is

doubtful whether this therapy altered the course of the disease itself in any way. However, since among the Japanese bronchopneumonia was a common complication after the lysis of the encephalitic fever, chemotherapy may have had some value in the prevention of secondary infection.

One of the most valuable therapeutic adjuncts was oxygen. Many observers have expressed the opinion that the pathologic changes demonstrable at autopsy are to a large degree contingent upon anoxia. The salutory effect of oxygen was seen definitively in one patient. Patient M. B., Case 2, was receiving intranasal oxygen. After his initial convulsion had been controlled with Sodium Amytal and intranasal oxygen had been started, no further convulsions occurred even in the absence of further sedation. At one period in the illness, oxygen was discontinued for thirty minutes because of a mechanical defect in the apparatus. At the end of this period of time, the patient became much more restless and more delirious and upon slight stimulation developed convulsive movements which threatened to culminate in a generalized grand mal type of attack. Intranasal oxygen was then reinstituted, and after fifteen minutes the patient could respond to some questions intelligently, the hyperirritability of the central nervous system had subsided, and even strong stimuli failed to elicit convulsive movements. Oxygen was again discontinued temporarily, this time purposefully, and in half an hour the patient had reverted to his former state of lethargy and hyperirritability. Thereafter oxygen was administered continuously, and the clinical response was swift and gratifying. It is interesting to note that oxygen was almost specific when there were absolutely no signs of circulatory distress. The chest and heart were normal clinically and roentgenologically, and no clinical cyanosis nor respiratory distress was apparent.

A second patient, Patient W. P., Case 3, already receiving oxygen, experienced recurrent convulsions and developed pulmonary edema. Intravenous Sodium Amytal controlled the convulsions, and a hypodermic injection of morphine controlled the pulmonary edema. Thereafter, with intranasal oxygen, in the absence of further sedation, the patient remained calm and free of convulsions. The role of oxygen in controlling the convulsions in this case is less clear-cut than in that of Patient M. B., Case 2, but it was difficult to escape the clinical impression that oxygen was at least partially responsible in the suppression of convulsions. In retrospect, oxygen probably should have been administered to all patients.

Headache was a very distressing symptom. Frequently the simple procedure of withdrawing 10 c.c. of spinal fluid for study ameliorated the headache considerably. All of the patients had slight elevations of the spinal fluid pressure. Aspirin and codeine were valueless. Some clinicians of the occupation forces employed intramuscular caffeine and sodium benzoate with good results. We had no experience with this method of treatment.

Incontinence of urine and feces was a problem only so long as the patient was comatose.

The remainder of the therapy was purely symptomatic and similar to that employed in any acute infectious disease. Respiratory and intestinal isolation

techniques were instituted, but it is dubious whether the disease is contagious via the respiratory route. Those persons entrusted with the care of encephalitic patients in Japanese hospitals practice no precautions and seem none the worse for their multiple exposures. Throughout Japan there are extremely few instances of the disease occurring in more than one member of a family. The necessity of adequate mosquito screening seems obvious.

Many of the features of the disease can be discerned readily by a brief study of the charts. Necessarily, from such a small series, no sweeping conclusions can be drawn. These charts represent only the clinical picture in five patients seen at this Dispensary. Good correlation has been obtained, however, with much larger series occurring among Japanese nationals throughout the empire.

Initially, all patients were febrile, the height of the elevation ranging from 102° F. to 105° F. plus. Definite lysis of the fever occurred on the seventh day of the illness in two patients, on the eighth and ninth days in two others, and on the tenth day in one. Temperature lysis about the seventh day of the illness is characteristic of Japanese B encephalitis. It is interesting to attempt to correlate the degree of the febrile response with the prevaling disturbance of the sensorium. Four patients had markedly disturbed sensoria. The fifth, Case 4, Patient J. S., with an equally high temperature and with the most marked spinal fluid pleocytosis, was mentally alert and cheerful throughout his illness.

Relative bradycardia was present initially in only two patients. This quickly disappeared.

The blood leucocyte count varied from 10,000 to 15,000 on admission, and in the majority of these counts there was a decided "shift to the left." The count returned to normal in three to seven days.

Pleocytosis in the spinal fluid ranged initially from 30 to 552 cells. In four patients there was a preponderance of polymorphonuclear cells. In the fifth, no differential was done on admission. However, the next day the majority of cells in the spinal fluid were polymorphonuclear. The ratio of neutrophilic leucocytes to mononuclear cells was soon reversed, and subsequent spinal fluid examinations revealed a decided majority of mononuclear cells. The Pandy reactions were positive, semiquantitatively, from a trace to two plus. There were no strongly positive Pandy reactions in any stage of the disease. Spinal fluid sugars and chlorides were normal in all instances. Repeated smears of the centrifuged spinal fluid were negative for organisms. No bacterial growth was obtained on repeated cultures. Because of the difficulty in following these patients after their discharge from the Dispensary and in order to avoid repeated lumbar punctures, it was impossible to ascertain exactly the length of time requisite for all abnormalities to disappear from the spinal fluid. In the five patients, definite abnormalities were found on the tenth, twentieth, twenty-first, thirty-second, and thirty-second days respectively.

During the febrile episode there was transient, mild proteinuria, but this finding disappeared upon lysis of the fever.

The neurological picture during the course of the illness has been described in the case histories. Nuchal rigidity was present in all, although the rigidity was quite mild. In only one patient, Case 3, Patient W. P., did a cranial nerve palsy develop (seventh nerve), although a transient weakness of the external (lateral) ocular rectus muscles was present in Case 3, Patient W. P., and in Case 2, Patient M. B. All except one, Case 4, Patient J. S., experienced gross tremors of the upper extremities, lips, and tongue. It is noteworthy that the Japanese physicians consider a tongue tremor to be one of the first signs of recovery. This observation was not well correlated with the clinical course in this series. The ocular fundi were normal in all patients. Reflex changes were varied and transient. At various stages of the illness there were hyperactive, hypoactive, and absent knee jerks, ankle jerks, and abdominal jerks. Kernig's sign was positive in some but not all patients. The Babinski was transiently positive in some. There was rapid disappearance of neurological reflex abnormalities after lysis of the fever. All patients, except Case 4, Patient J. S., exhibited a lead-pipe type of rigidity of the upper extremities. The sensoria of all patients, except Case 4, Patient J. S., were markedly deviated from normal. These patients were restless and aggressive, with alternate periods of lethargy. This is in marked contrast to the extreme constant lethargy usually prevelant among native encephalitis victims.

One characteristic of the disease which deserves special emphasis is the rapidity of the patient's recovery. He may appear moribund, and the outlook may seem virtually hopeless. Even in such instances recovery may be swift.

There were no fatalities in this small series. The Japanese mortality rate of approximately 35.7 per cent is undoubtedly excessively high because of the poor methods of treatment in their hospitals. Even during the acute stage, while the patient is comatose, only 500 c.c. of fluid are administered each day by intramuscular drip, according to the practice in one of their large hospitals. This amount is not varied, regardless of the degree of dehydration, age, or size of the patient. "Acidosis" is "prevented" by the intravenous administration each day of 20 c.c. of a 50 per cent glucose solution. Oxygen, apparently, is infrequently, if ever, employed. A further disadvantage is the marked overcrowding of their hospitals with encephalitic patients, rendering very difficult the task of individualized attention by the staff of doctors.

Two patients in this series developed minor physical sequelae. In one, Case 1, Patient T. H., there was a definite change in the voice. The patient developed a very hoarse voice which improved after two months. No paralysis of the vocal cords has been demonstrated. The possibility of weakness of the superior laryngeal nerve was entertained. A second man, Case 3, Patient W. P., developed weakness of the adductor of the left thumb and flexors of the left forefinger. Initially, sensory perception was lost over the dorsal and palmar aspects of the second and third fingers of the left hand. The sensory abnormalities disappeared, leaving the residual muscle weakness outlined. Physiotherapy has greatly improved this condition.

The possible permanent mental changes resulting from infection with the encephalitic virus would comprise a most interesting and fruitful field for study. Limitations of time and lack of trained psychiatric consultants pre-

vented a detailed investigation of this important aspect of the disease. Lack of knowledge concerning the behavioristic pattern of the patient prior to his illness represents an intrinsic impediment in a study of this type. Without proper controls, only gross abnormalities can be attributed to the disease. None of these patients exhibited any marked impairment of the process of cerebration. Their intellectual ability was well within the normal range when tested by simple measures such as arithmetic problems, ability to exercise judgment in simple situations, and general knowledge.

Emotionally a similar pattern of dependence was evident in three of them. Even when the storm of the illness had passed and the patients were alert and recuperating remarkably, there was a strong reluctance on their part to attend in any way to their own needs. Only with difficulty, with the exception of one patient (Case 1, Patient T. H.), were they persuaded to abandon their recumbent positions. They were very loath to accept again the responsibility of bathing themselves. One patient, Case 2, Patient M. B., with a probable spondylitis, was extremely happy in his prolonged convalescence and almost refused to attempt anything for himself except the actual eating of his meals. However, the remainder of the patients did bow to the inevitable and, with one exception, Case 5, Patient M. W., were eager to return to their occupations. Case 5, Patient M. W., was anxious to return home provided she could convalesce for an indefinite period. Later it was ascertained that she promptly installed herself in bed at home and there remained for six weeks. The patient manifested extreme dependency, emotional lability, and was excessively demanding of those who attended her. According to one of her friends, she had definitely "changed" since her illness. Lapses in recent memory became obvious. Upon one occasion while entertaining guests, her husband left the room for a few moments. Almost coincident with his departure his wife confided in a friend her anxiety concerning him, as he had failed to return home that evening and had not called. Obviously, with no adequate preconfinement studies personality tendencies cannot indisputably be ascribed to the Japanese B virus.

One man, Case 1, Patient T. H., who least displayed this type of personality dependence, developed a curious propensity for inserting himself into odd and dangerous situations. He had a definite proclivity for wandering through the women's ward at night. He was content to confine his wanderings to the corridors and never did he enter any of the rooms. He had no explanation for his behavior. In one instance, he stopped the elevator between floors, climbed on top, and was reclining comfortably when discovered. Again, there was no adequate explanation. However, these bizarre quirks of behavior disappeared rapidly, and the patient was quite embarrassed when they were recalled to his attention. Interestingly enough, these peculiar phenomena occurred when the man seemed alert and well oriented. It seems possible that the encephalitic damage occasioned these transitory abnormalities.

All of the convalescent patients were unusually cheerful. Even when residues such as facial nerve palsy or tremors were evident, the patients ignored them magnanimously and refused to allow themselves to sink into the depths of depression. Only one patient, Case 5, Patient M. W., was emotionally labile,

but even she usually maintained a cheerful equilibrium. This emotional lability persisted in her for approximately six weeks after discharge.

The routine course of immunization comprised the administration of 1 c.c. of the chick embryo vaccine at weekly intervals for two doses followed by an additional 1 c.c. one month later. Case 4, Patient J. S., had only two injections; Case 2, Patient M. B., had none; Case 1, Patient T. H., had completed his course of immunization; Case 3, Patient W. P., had been immunized in 1946 and had received a booster dose of 1 c.c. in 1947 and 1948; Case 5, Patient M. W., had received no immunization.

## SUMMARY

1. A series of five serologically proved cases of Japanese B encephalitis is presented. Six other patients with the same signs and symptoms were hospitalized during the epidemic but are not included in the series as no positive serologic proof could be obtained.

2. Only two of the five patients had completed their immunizations against Japanese B encephalitis.

3. The clinical features are outlined.

4. In addition to rehydration and sedation when necessary, oxygen seems to be a valuable therapeutic adjunct.

5. Only two minor physical sequelae occurred in five cases. There were no deaths.

6. The possibility of personality changes, resulting from the encephalitic virus, is discussed.

7. Improved treatment will probably reduce considerably the high mortality rate for Japanese B encephalitis prevalent among the Japanese.

The author wishes to express his appreciation to the Virus and Rickettsial Section, 406th Medical General Laboratory, Tokyo, Honshu, Japan, for the determination of complement fixations and neutralization indices.

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# PANCREATITIS IN INFECTIOUS MONONUCLEOSIS

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PANCREATITIS in association with mumps is often mentioned, though the literature on this point is not conclusive. Pancreatitis has been demonstrated definitely, however, in three patients with mumps, one at necropsy[1] and two at the time of surgical exploration.[2, 3]

There are few autopsy reports of cases of infectious mononucleosis. In eight case reports[4, 5, 6, 7] the pancreas was not mentioned. In three case reports[8, 9, 10] the pancreas was described as normal. There seems to be general agreement that infectious mononucleosis is a disease which may affect many organs.

Lucke and Mallory,[11] in their excellent study of the pathology of hepatitis, did not mention the pancreas. Capps,[12] however, mentions lesions occurring in the intestinal tract and in the pancreas. Comfort[13] noted in one case a persistent steatorrhea after infectious hepatitis and speculated on the association of pancreatitis with hepatitis.

In order to explore further the possibility that pancreatitis may be associated with other viral diseases, determinations of serum amylase and lipase were made at approximately weekly intervals on twenty consecutive patients with infectious mononucleosis and nine patients with viral hepatitis.

## MATERIALS AND METHODS

Serum amylase was determined by the Somogyi method.[14] Incubation times were determined to the nearest minute. The range of error in duplicate and triplicate determinations of the same specimen of normal serum was 0 to 36 per cent with a mean error of 6 per cent. Serum lipase was determined by the Cherry-Crandall[15] method as modified by Maclay.[16] Titrations were done with 0.1N NaOH to the nearest 0.1 cubic centimeter. The range of error on duplicate and triplicate determinations on the same specimen of normal serum was 0 to 50 per cent with a mean error of 14 per cent. The mean control amylase value based on thirty-six normal subjects was 146.4 units with a standard deviation of 55.7. Using three times the standard deviation as a criterion, the upper limit of normal for this group would be 313.5 Somogyi units. The mean control lipase value based on thirty-seven normal subjects was 0.27 c.c. with a standard deviation of 0.16. Using three times the standard deviation as a criterion, the upper limit of normal for this group would be 0.75 c.c. of 0.1N NaOH.

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Abstract of a portion of the thesis submitted by Dr. Myhre to the faculty of the Graduate School of the University of Minnesota School of Medicine in partial fulfillment of the requirements for the degree of Master of Science in Internal Medicine.

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Received for publication, Aug. 19, 1949.

## RESULTS

Two of the twenty patients with infectious mononucleosis presented abnormal serum enzyme values. In one patient values for both serum amylase and serum lipase were elevated, the highest serum amylase being 450 Somogyi units and the highest serum lipase being 1.7 c.c. of 0.1N NaOH. The second patient had abnormal values of serum lipase, the highest being 1.0 cubic centimeter. In this instance the values for serum amylase, although within the normal range, increased from 108 to 228 units. Liver function tests were done in nineteen of these patients and suggested an associated hepatitis in eighteen.

In nine patients with viral hepatitis, similar enzyme determinations at various stages of the disease gave normal results in all instances. Three were considered to be of the infectious type and six to be of the homologous serum variety.

Because of their particular interest, the two patients with infectious mononucleosis who, during the course of their illness, exhibited elevated serum enzyme values suggestive of an associated pancreatitis are presented in some detail. (Table I.)

CASE 1.—A 24-year-old, white, male school teacher, who had been in good health previously, was admitted to the hospital Jan. 3, 1948, complaining of headache and malaise of fourteen days' duration and of sore throat and fever up to 103° F. for one week. Penicillin and sulfadiazine had been administered by his family doctor without benefit. Physical examination revealed an acutely ill patient, the temperature being 103.4° F., and the presence of cervical, inguinal, and axillary adenopathy, marked tonsillitis, hepatomegaly, and splenomegaly. The hemoglobin concentration was 12.6 grams per 100 c.c. of blood and the leucocytes numbered 20,800 per cubic millimeter of blood. The percentages of the various types of leucocytes were as follows: neutrophils 30, lymphocytes 66 (many of them atypical), monocytes 2, basophils 1, and eosinophils 1. The Kahn test was 2 plus. Albuminuria was graded 2 plus and microscopic examination of the urine sediment revealed 3 to 6 leucocytes, occasional erythrocytes, hyaline casts, and granular casts per high-power field. Roentgenogram of the chest revealed moderate accentuation of the broncho-vascular markings bilaterally and a faint suggestion of pneumonitis in the left lower lung field. Bromsulfalein retention was 10.5 per cent at 45 minutes using a dose of 5 mg. per kilogram per body weight. The result of the cephalin flocculation test was 3 plus in 24 hours and 3 plus in 48 hours. The one-minute serum bilirubin was 0.2 mg. per cent and the total was 0.5 mg. per cent. Predominantly hemolytic staphylococci grew from a throat culture.

A clinical impression of infectious mononucleosis was corroborated by the relative and absolute lymphocytosis, the appearance of atypical lymphocytes in the peripheral blood smear, and a strongly positive heterophile antigen-antibody agglutination titer. The laboratory studies are presented in detail in Table I.

On the thirty-first day of illness the serum amylase was 450 units and the serum lipase was 1.7 cubic centimeters. In approximately twenty days these values had returned to normal. A glucose tolerance curve at the height of the elevation of serum amylase and lipase was normal.

Treatment consisted of bed rest, administration of 50,000 units of penicillin intramuscularly every three hours for the five days after admission, a high carbohydrate, low fat, and high protein diet with vitamin supplements and added brewers' yeast. The Kahn and Kolmer-Wassermann tests were negative after the patient had been two days in the hospital. The abnormal urinary findings disappeared after one week. A roentgenogram of the chest, taken ten days after admission, was normal. One week after penicillin therapy was discontinued a maculopapular rash appeared over the trunk. The liver was enlarged and tender for approximately one month after admission. There was never any abdominal

TABLE I. DETAILED CLINICAL AND LABORATORY DATA OF THE TWO PATIENTS WITH INFECTIOUS MONONUCLEOSIS WHO HAD ELEVATED VALUES OF SERUM AMYLASE AND LIPASE

| DATE (1948) | DAY OF DISEASE | TEMPERATURE | RASH | TONSILLITIS | W.B.C. × 1,000 | PER CENT POLYS | PER CENT LYMPHOCYTES | HETEROPHILE TITER | CEPHALIN FLOCCULATION | SERUM BILIRUBIN | BROMSULFALEIN (%) | KAHN | SERUM AMYLASE | SERUM LIPASE |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *Case 1* | | | | | | | | | | | | | | |
| Jan. 3 | 15 | 103 | – | 3+ | 12.9 | 31 | 66 | 1:1,792 | | | | | | |
| Jan. 6 | 18 | 102 | – | 2+ | 20.8 | 30 | 66 | | | | | 2+ | | |
| Jan. 8 | 20 | 101 | – | 2+ | | | | | 3+ 3+ | | | | | |
| Jan. 11 | 23 | 100 | – | 1+ | 16.5 | 10 | 86 | 1:1,792 | | | | Neg. | | |
| Jan. 15 | 27 | 99 | + | – | | | | | | | 10.5 | Neg. | | |
| Jan. 21 | 31 | 98.6 | + | – | 9.4 | 23 | 75 | | 3+ 3+ | .2–.5 | | | 450 | 1.7 |
| Jan. 23 | 33 | 98.6 | + | – | | | | | | | | | 400 | 1.3 |
| Jan. 26 | 36 | 98.6 | – | – | 8.4 | 26 | 73 | 1:1,792 | | | | | | |
| Jan. 28 | 38 | 98.6 | – | – | | | | | | | | | | 1.4 |
| Jan. 29 | 39 | 98.6 | – | – | | | | | | | | | 200 | 1.2 |
| Feb. 4 | 45 | 98.6 | – | – | | | | | | | | | 246 | 1.0 |
| Feb. 10 | 51 | 98.6 | – | – | | | | | | | 1.2 | | | 0.7 |
| Feb. 25 | | 98.6 | – | – | 9.7 | 32 | 53 | | | | 1.5 | | 130 | |
| *Case 2* | | | | | | | | | | | | | | |
| Apr. 4 | 5 | 102 | – | – | 8.0 | 61 | 34 | | | | | | | |
| Apr. 6 | 7 | 102 | – | – | 7.3 | 47 | 44 | 1:448 | | | | Neg | | |
| Apr. 8 | 9 | 102 | – | 1+ | 11.7 | 29 | 65 | | 3+ 3+ | .2–.5 | | | 108 | |
| Apr. 12 | 13 | 103 | – | 2+ | 16.8 | 30 | 60 | 1:896 | 3+ 4+ | .1–.5 | | | | |
| Apr. 14 | 15 | 102 | – | – | | | | | 3+ 4+ | | 11 | | | |
| Apr. 19 | 20 | 100 | – | – | | | | | | | | | 158 | |
| Apr. 21 | 22 | 98.6 | – | – | | | | | | | | | | 1.0 |
| Apr. 22 | 23 | 98.6 | – | – | | | | | | | | | | 0.9 |
| Apr. 23 | 24 | 98.6 | – | – | | | | | | | | | 228 | 0.6 |
| Apr. 26 | 27 | 98.6 | – | – | | | | | | | | | 200 | |
| Apr. 28 | 29 | 98.6 | – | – | | | | | | | | | 190 | 0.5 |
| June 10 | | 98.6 | – | – | | | | | 0 1+ | | 1 | | 120 | |

pain or diarrhea but occasionally the patient complained of backache. Appetite and strength gradually returned and the lymphadenopathy decreased. During the course of the illness the patient lost 23 pounds in weight.

CASE 2.—A 21-year-old, white student nurse who had previously been in good health was admitted to the hospital April 4, 1948, complaining of a slight cold and nasal congestion of five days' duration. Two days before admission she had noticed fever, fatigue, chilly sensations, and loss of appetite. Physical examination revealed an acutely ill patient with a temperature of 102° F. There was a slight mucoid nasal discharge. The throat was normal in appearance. The heart and lungs were within normal limits. A slightly enlarged, tender lymph node was found in the right anterior cervical region. The liver and spleen were not enlarged and there was no rash.

The hemoglobin concentration was 12.6 grams per 100 c.c. of blood and the leucocytes numbered 8,000 per cubic millimeter. The percentages of the various leucocytes were as follows: neutrophils 61, lymphocytes 34, monocytes 3, and eosinophils 2. The Kahn test for syphilis was negative. The urinalysis did not reveal abnormalities. Roentgenogram of the chest was normal. *Streptococcus viridans* and *Neisseria catarrhalis* grew from a throat culture. The heterophile antigen-antibody titer was 1:448. The cephalin flocculation test gave 3 plus results in 24 hours and 48 hours. The one-minute and total serum bilirubin concentrations were 0.2 and 0.5 mg. per cent respectively and the bromsulfalein retention with a dose of 5 mg. per kilogram of body weight was 11 per cent. Alkaline phosphatase was 10 King-Armstrong units. The prothrombin time was 44 per cent of normal. Total proteins were 6.2 grams per cent with 3.3 grams per cent of albumin and 2.9 grams per cent of globulin. The blood cholesterol was 134 mg. per 100 c.c. of serum with 42 per cent cholesterol esters. Bleeding time, clotting time, and platelet counts were normal. Two routine blood cultures and one blood culture with increased carbon dioxide tension were sterile. The blood was type O and Rh negative.

On the ninth day of illness the serum amylase was 108 Somogyi units and on the twentieth day, 158 units. On the twenty-second day the serum lipase was 1.0 c.c. of 0.1N NaOH and on the twenty-third day was 0.9 cubic centimeter. On the twenty-fourth day the serum amylase and lipase were 228 units and 0.6 c.c. respectively. Subsequent values for serum lipase rapidly returned to normal and the serum amylase decreased to 120 units. At this time the fasting blood sugar was 96 mg. per cent and the urinalysis for sugar was negative.

Subsequent development of an absolute lymphocytosis, the appearance of atypical lymphocytes in the stained peripheral blood smear, and a strongly positive heterophile antigen-antibody titer established the diagnosis of infectious mononucleosis. See Table I for complete details of the laboratory studies.

On the tenth day of disease numerous petechiae were noted on the hard palate which persisted approximately four days. About the same time the normal pharynx became injected in appearance, the tonsils were covered by a grayish-white membrane, and at this time the predominant organism on throat culture medium was nonhemolytic streptococcus. The sore throat accompanied by headache and pain on moving the eyes had nearly disappeared when, on the fifteenth day of illness, the patient became markedly anorexic and nauseated and vomited repeatedly. Treatment at this time entailed administration intravenously of solutions containing glucose, Parenamine, B complex vitamins, ascorbic acid, and Synkayvite. The liver became tender to percussion but still was not enlarged. The patient became afebrile on the twenty-second day of illness and by the twenty-ninth day she was sufficiently improved to be discharged to her home for gradual resumption of activities. She had lost at least 8 pounds in weight.

## SUMMARY AND CONCLUSIONS

1. Twenty consecutive cases of infectious mononucleosis were studied at weekly intervals and in two instances a definite elevation of the values of serum amylase and lipase was demonstrated. This finding is suggestive of an as-

sociated pancreatic disturbance which to our knowledge has not been described previously in patients with infectious mononucleosis. This was possibly a pancreatitis, but lymph node obstruction of the pancreatic ducts must be considered.

2. In nine cases of viral hepatitis the serum amylase and lipase determinations were made at varying intervals and no elevated values were found. No conclusions can be drawn from this small series except that there was no evidence for pancreatitis at the time the determinations were made. This series should be extended and the possibility of pancreatitis occurring in other viral diseases should be explored.

3. It is quite possible that many asymptomatic, acute, subacute, and chronic inflammatory changes of the pancreas exist in association with viral diseases which at the present time are not appreciated. It is conceivable that such infection may play a role in the etiology of chronic relapsing pancreatitis, the pathogenesis of which remains obscure.

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# A CASE OF CONGENITAL IDIOPATHIC METHEMOGLOBINEMIA

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THE differential diagnosis of cyanosis in the adult does not often present a difficult problem except in the instance of chronic, persistent cyanosis which does not vary. This is more interesting if the patient is middle aged or older, and if the cyanosis has been present during the greater part of his life. Proper consideration must be given to cyanosis produced by altered hemoglobin compounds within the circulating blood. The nature of the compound and its concentration in the blood are important in the diagnosis, treatment, and prognosis of these disorders. The three pigments (always intracorpuscular) which most frequently produce cyanosis are: reduced hemoglobin (as in cardiopulmonary disease), methemoglobin, and sulfhemoglobin. When these are present in the blood in concentrations of 5, 1.5, and 0.5 Gm. or less per 100 ml. of blood, respectively, cyanosis may be clinically evident.[1] Minute amounts of methemoglobin (0.03 to 0.13 Gm. per 100 ml.) have been reported in the blood of normal persons.[2] This may be a normal stage in the degradation of the pigment. The compounds have several definite chemical and physical properties by which they may be identified.[3]

Clinically, methemoglobinemia may be primary or secondary. The secondary type is more common and is most often due to drug toxicity. Anemia and/or granulocytopenia are usually accompanying findings in chronic cases, and the systemic effects are striking. Of much less frequent occurrence is the primary type. There is no anemia; indeed, a polycythemia is often present apparently as a compensatory measure. Systemic symptoms are exceptionally mild, and a familial incidence has been noted in many of the cases reported. Since the condition is rare, the following case is believed to be of interest.

## CASE REPORT AND EXPERIMENTAL STUDY

A 61-year-old white man was admitted to the Marine Hospital with complaints of failing vision in both eyes. On physical examination, bilateral cataracts were discovered. It was also noted that the patient had a marked cyanosis of the mucous membranes, lips, and fingers, and a slate gray color of the skin all over the body. There was no clubbing of the digits, and the conjunctival and retinal blood vessels were engorged. The patient stated that his blue-gray skin coloring had been present "since birth," and that people had frequently remarked about it when he was a child. No other familial instance could be elicited. There was no history of prolonged drug ingestion, and system review failed to disclose any abnormality of gastrointestinal function. The patient had no complaints other than his visual disturbance, and cardiopulmonary disease was ruled out by physical examination, x-ray and fluoroscopic studies, and normal electrocardiographic tracings.

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Received for publication, Aug. 29, 1949.

The patient was seen in consultation by Dr. A. J. Beams,* who suggested a diagnosis of idiopathic methemoglobinemia. A sample of blood was examined and found to contain: total hemoglobin, 21.9 Gm. per 100 ml.; oxyhemoglobin, 14.3 Gm.; and the difference, 7.6 Gm., was nonfunctional hemoglobin which showed a spectroscopic absorption band similar to that of a known methemoglobin solution. Laboratory findings at this time revealed nothing of significance except an icterus index of 17.6 units; red blood cells, 6,350,000 per cubic millimeter; and a hematocrit of 68 per cent packed red cells. The red cell indices revealed a mean corpuscular volume of 106 c.micra and mean corpuscular hemoglobin concentration of 29.2 per cent. The white blood cells were normal.

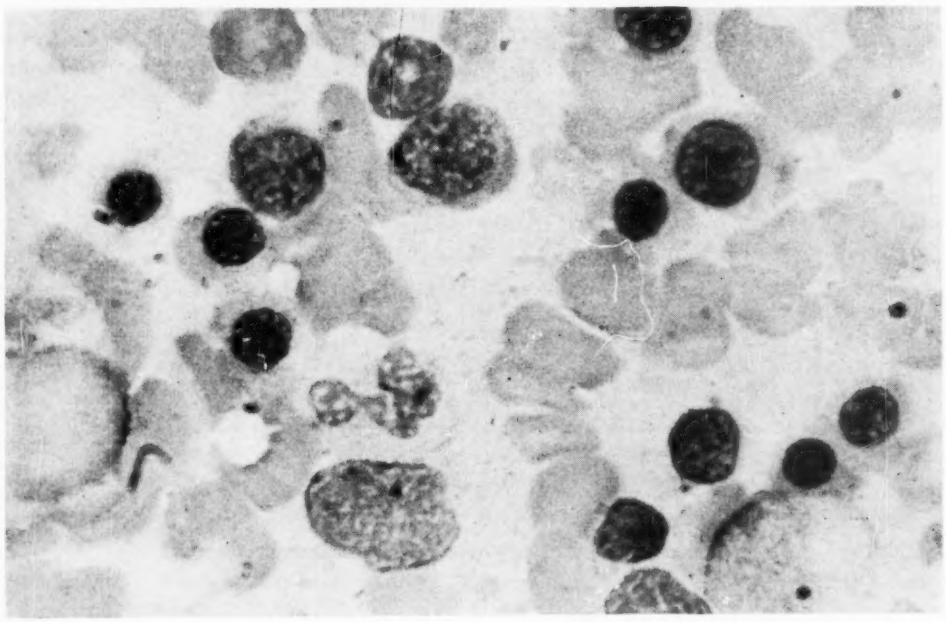

Fig. 1.—Sternal bone marrow (×950) showing normoblastic hyperplasia.

The patient was operated on for a cataract of the left eye and then discharged from the hospital. Four months later he was asked to return to the hospital and was admitted to the medical service for further study of his methemoglobinemia. On this second admission he had no complaints and was not dyspneic. The physical examination was essentially the same as on the first admission except for a partial left iridectomy and absence of that lens. The blood pressure was 150/94, temperature 96.2° F., pulse 80, and respirations 24. The red blood cell count was 5,930,000 per cubic millimeter, with a hemoglobin of 18.9 grams. The hematocrit was 61 per cent. When venous blood was drawn it had a dark, chocolate-brown color. Exposure of the blood to air over a period of twenty-four hours produced no noticeable change in color, and long periods of oxygen inhalation did not affect the patient's cyanosis. The reticulocytes numbered 0.7 per cent, and the icterus index was 13.0 units. The total hemoglobin measured 19.9 Gm., oxyhemoglobin, 12.5 Gm., and methemoglobin, 7.4 Gm. per 100 ml. of blood prior to the beginning of therapy. Aspiration of sternal bone marrow yielded a moderately thick marrow which was also chocolate-brown in color. The centrifuged marrow showed fat, 5 per cent; plasma, 32 per cent; buffy (myeloid: erythroid) layer, 9 per cent; and red cells, 53 per cent. Marrow smears showed an erythroid hyperplasia at the normoblast level. (Fig. 1.)

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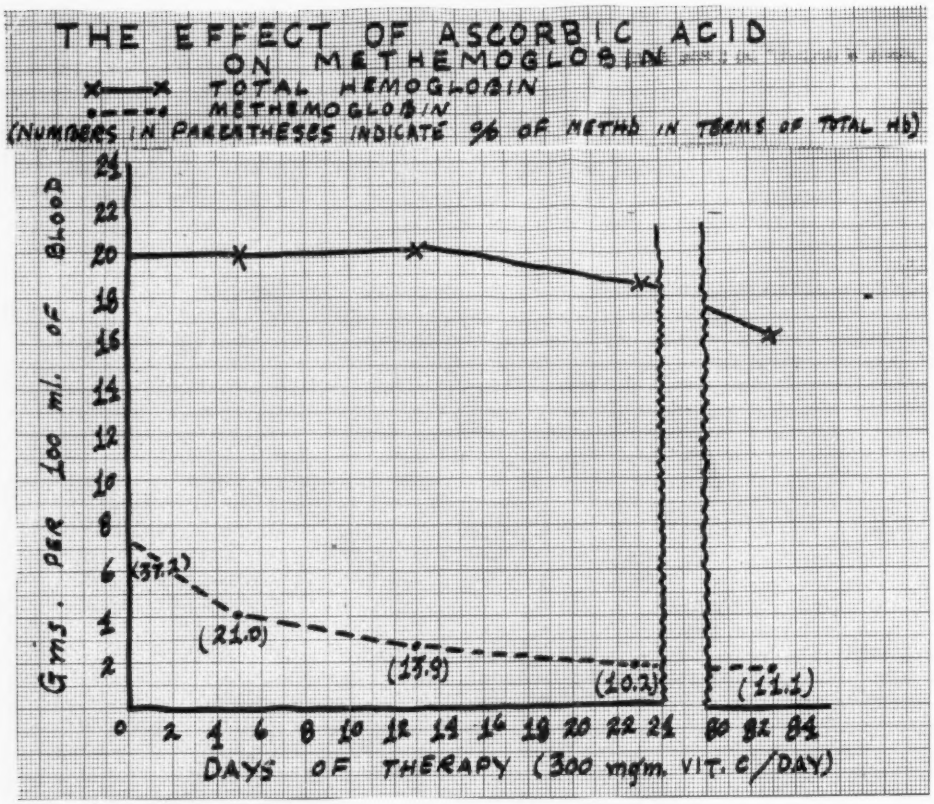


Fig. 2.—The effect of ascorbic acid on methemoglobin.

The patient was then started on oral treatment with ascorbic acid in doses of 300 mg. each day. Clinical improvement was slow at the start but easily noticeable by the third day of therapy. On the fifth day the patient noted the change in his color, and the venous blood appeared more red. At the end of ten days, the labial and gingival cyanosis was almost gone. By the end of the second week, the total blood methemoglobin had been reduced to almost one-half (Fig. 2). The laboratory findings during this therapy are summarized in Table I; the changes in methemoglobin are shown in Fig. 2.

This therapy was continued for one month, during which time the patient's skin color became more pink. The venous blood appeared almost normal in color, and the methemoglobin level dropped to 1.9 Gm. per 100 milliliters. By the end of this time, the patient

TABLE I. LABORATORY DATA DURING PERIOD OF THERAPY

| | DAY OF THERAPY | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 0 | 3 | 6 | 10 | 13 | 17 | 20 | 83 |
| R.B.C. (millions) | – | 6.88 | 6.31 | 7.19 | 5.97 | 6.20 | 6.87 | 5.35 |
| Total Hb. (Gm. per 100 ml.) | – | 21.1 | 18.9 | 20.9 | 19.5 | 19.5 | 19.5 | 17.0 |
| Hematocrit (% packed red cells) | 69.0 | 68.0 | 66.5 | 69.0 | 63.0 | 62.5 | 62.0 | 52.0 |
| Icterus index (units) | 13.6 | 9.2 | 8.6 | 7.7 | 10.4 | 12.9 | 11.6 | 13.6 |
| Reticulocytes (%) | 0.7 | – | 0.8 | – | 0.7 | 0.3 | 0.3 | 0.5 |
| Urine: Bilirubin negative in all specimens; urobilinogen present in normal amounts | | | | | | | | |

wished to be discharged from the hospital. He was placed on outpatient status and given a supply of ascorbic acid, but he failed to return for follow-up. Two months later he returned for more ascorbic acid, and further data were then obtained. His skin color was definitely a normal pink hue, and the venous blood had a normal dark red color. The laboratory findings at this time are listed as "83rd day" in Table I and Fig. 2. The bone marrow showed no significant change.

## DISCUSSION

This case was of interest for several reasons: the chronicity of the illness, the high initial methemoglobin level, and the response during the period of therapy. The patient was able to carry on strenuous work over a period of years, as a merchant seaman, without dyspnea, fatigue, or other constitutional symptoms. Finch,[1] who reviewed this subject exhaustively in 1948, stated that he saw a patient with a methemoglobin level of 40 per cent who was able to carry on strenuous work; our patient had 37.2 per cent. A more remarkable point of interest arose during the course of therapy. Although the usual response with treatment is a reduction in methemoglobin and total hemoglobin, our patient exhibited a different course. As the methemoglobin decreased, the total hemoglobin remained stationary and the oxyhemoglobin level rose. The red cell count and hematocrit also remained fairly stationary. This was entirely contrary to our expectations, since we anticipated hemolysis of the excess erythrocytes as the methemoglobin was reduced. Undoubtedly the chronic anoxemia present for so many years proved a potent stimulus to the erythrogenic elements of the bone marrow. Had we been able to follow the patient for a longer period, it would have been interesting to note how long a time elapsed before the number of circulating red cells became reduced in number.

At least sixteen proved cases of congenital idiopathic methemoglobinemia had been reported in the literature up to the time of Finch's review (1948). In eight, there was a familial incidence. In 1947, Coudounis and associates[4] reported fourteen cases, all occurring in a Greek family within four consecutive generations; four pairs of siblings were affected by the syndrome. The name of the family was Vophtochilari, which the authors translated as "people with discolored lips." Sievers and Ryon[5] stated that they were able to tabulate eighteen reported cases up to 1945, and they added one of their own. Gibson and Harrison (1947)[6] reported five cases in one family. Bensley, Rhea, and Mills (1938)[7] reported two cases occurring in a brother and sister and stated that these were the first in which the diagnosis was established definitely. They added that the cyanosis was constant and was not affected by any form of therapy; vitamin C was not used.

Other cases have been reported in the literature from time to time. In some, history of drug intoxication could not be ruled out. Others were given the name of "enterogenous cyanosis," supposedly associated with constipation and production of large amounts of nitrites by the bacteria of the intestinal tract.

To Lian[8] is ascribed the first use of ascorbic acid in the treatment of methemoglobinemia. It was believed to be superior to methylene blue. Ascorbic

acid has been used in many of the cases reported since then, almost all with excellent results.[10] The oral route is as effective as the parenteral, and Graybiel and co-workers[9] have found that concomitant administration of sodium bicarbonate decreases the urinary excretion of vitamin C and the rate at which it disappears from the blood. King, White, and Gilchrist[10] found that regardless of the amount of ascorbic acid given to their patient, the methemoglobin level could not be reduced below 7 per cent of the total blood pigment. Gibson and Harrison[6] also found that they were not able to reduce the total methemoglobin below 1.0 Gm. per 100 ml. of blood by the use of vitamin C. Our findings, over a short period of time, are similar to these.

Normally, the mammalian anuclear erythrocyte reduces methemoglobin by an enzymatic process in which glucose and lactate are the principal substrates. Clinical methemoglobinemia may then be produced by absence or failure of this reconversion mechanism (primary) or by the action of oxidants which produce methemoglobin more rapidly than the cell is able to reduce it (secondary). Further, the oxygen dissociation curve is normal in primary methemoglobinemia, whereas that induced by drugs shows a shift to the left.[1]

Gibson proposes that the erythrocytes of primary methemoglobinemic patients are deficient in coenzyme factor I, which functions with triose phosphoric and lactic enzymes of the red blood cell to facilitate reduction of methemoglobin to hemoglobin. Methylene blue is therefore thought to catalyze reduction of methemoglobin by enzymes otherwise unable to bring about this reaction. Ascorbic acid, by contrast, reduces methemoglobin directly, but is never complete.[6]

These differential diagnostic features are important in providing the proper therapy for methemoglobinemia.

## SUMMARY

A case of congenital idiopathic methemoglobinemia in a 61-year-old man is presented. The patient was treated with daily oral doses of 300 mg. of ascorbic acid and showed an excellent, though somewhat unusual, response to therapy. The case is of interest because of the age of the patient, the chronicity of the disease, and the trend during therapy. Some of the pertinent literature is reviewed briefly.

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# *LABORATORY METHODS*

## A SIMPLIFIED TURBIDIMETRIC METHOD OF AUREOMYCIN ASSAY FOR CAPILLARY BLOOD AND OTHER BODY FLUIDS

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FROM studies on various methods of bio-assay for aureomycin, a turbidimetric method has evolved which is sufficiently accurate and sensitive in the presence of body fluids for investigative purposes and is sufficiently simple for routine use. It also meets a prime requirement for pediatric use in that an amount of blood adequate for serum assay can be obtained from a single finger or heel puncture. Osgood and Graham[1] developed a similar turbidimetric method of assay for penicillin, streptomycin, and trivalent organic arsenicals. Meads and co-workers[2] adapted it to the assay of aureomycin. We found that for aureomycin the accuracy of this method in the presence of body fluids is increased appreciably by substituting a standard curve containing four points for the single standard used by the original workers and that this change also simplifies the method by eliminating most of the mathematical details involved in computing the results.[3] Several other minor modifications were also helpful. It therefore seems worth while to publish the details of this method as it is being used in our laboratory.

### DETAILS OF TECHNIQUE

*Materials.*—A Klett-Summerson colorimeter with a No. 660 filter; sterile matched colorimeter tubes with rubber stoppers, 0.85 per cent saline, 1 and 5 c.c. pipettes; a 37° C. water bath, an ice water bath in the refrigerator, and standardized, uncontaminated test organism (Oregon J strain of *Staphylococcus aureus*—American Type culture collection No. 9801) are used.

The organism is standardized as follows: 5 ml. amounts of a fresh one- to three-hour broth culture* are added to 100 ml. amounts of sterile Bacto-Tryptose Phosphate Broth (Difco), which is then incubated in the 37° C. water bath until the optical density of the culture as determined by colorimeter readings at fifteen-minute intervals is approximately fifteen points (twelve to eighteen points) greater than that of a broth blank. This usually takes two and one-half to three hours. At this point, growth is stopped by transferring the culture to the ice water bath. This, the standardized culture, is kept in the ice water bath until used and is made up fresh every four days.

From The Children's Hospital of Philadelphia (Department of Pediatrics, School of Medicine, University of Pennsylvania).

The aureomycin used in this study was supplied by Lederle Laboratories Division of the American Cyanamid Company.

These studies were conducted under contract with the Department of the Army, Chemical Corps, Camp Detrick, Maryland.

Received for publication, Aug. 1, 1949.

*This is prepared by inoculating Bacto-Tryptose Phosphate Broth from the stock culture. The stock culture is kept on a nutrient agar slant in the refrigerator. A fresh slant is prepared every two weeks. Each new slant is checked for contamination before use.

A series of previously matched, cotton-plugged, sterile colorimeter tubes is arranged for assay as shown in Table I. As soon as all dilutions are made, sterile rubber stoppers are inserted into the tubes and the solutions agitated by inversion of the tubes. The colorimeter is set at zero with the blank tube, and the turbidity of each remaining tube is read rapidly in a predetermined order. Immediately after reading, each tube is placed in the 37° C. water bath. Four hours later each tube is individually removed from the water bath, wiped, agitated, and reread in the same order.

TABLE I. MATERIALS ADDED TO TUBES FOR TURBIDIMETRIC ASSAY OF AUREOMYCIN IN BODY FLUIDS

| TUBE | APPROPRIATE BODY FLUID* (ML.) | SALINE (ML.) | MICROGRAMS OF AUREOMYCIN PER ML. OF SALINE† | | STANDARDIZED TEST CULTURE‡ (ML.) |
|---|---|---|---|---|---|
| | | | FOR URINE ASSAY | FOR SERUM AND SPINAL FLUID ASSAY | |
| Blank | 0 | 5.0 | 0 | 0 | 0 |
| Control | 0.5 | 0.5 | 0 | 0 | 4.0 |
| Standard 1 | 0.5 | 0.5 | 2.0 | 1.0 | 4.0 |
| 2 | 0.5 | 0.5 | 1.0 | 0.5 | 4.0 |
| 3 | 0.5 | 0.5 | 0.5 | 0.25 | 4.0 |
| 4 | 0.5 | 0.5 | 0.25 | 0.125 | 4.0 |
| Unknown 1 | 0.5 | 0.5 | 0 | 0 | 4.0 |
| 2 | 0.5 | 0.5 | 0 | 0 | 4.0 |
| Etc. | 0.5 | 0.5 | 0 | 0 | 4.0 |

*Contains no added aureomycin.

†Aureomycin dilutions freshly made from a 5,000 μg per milliliter of aureomycin solution, which is made up fresh every two weeks from lyophilized aureomycin and kept at 4° C.

‡Added last while still at 4° C.

Computation of the results hinges on the principle demonstrated by Osgood[1] that, in the case of a sensitive organism, when sufficient bacterial growth has occurred to provide for differences that can be accurately measured, the concentration of the appropriate antibiotic is proportional to the square of the difference between the turbidity of a control tube and the turbidity of a tube containing the antibiotic. The details are described as follows and are illustrated in Table II. The increase in turbidity between the four-hour reading and the zero-hour reading is determined in each of the four standard tubes (containing known amounts of aureomycin), each of the unknown tubes (containing the body fluid to be assayed), and the control tube (containing no aureomycin). The increase in turbidity (which is the measurement of bacterial growth during the four hours of incubation) of each standard tube and each unknown tube is then subtracted from the increase in turbidity of the control tube. The difference in turbidity increase between the control

TABLE II. COMPUTATION OF RESULTS FOR A SERUM STANDARD CURVE

| TUBE | AUREOMYCIN (γ PER ML.*) | TURBIDITY IN KLETT UNITS | | TURBIDITY INCREASE (4 HR.-0 HR. READING) | DIFFERENCE IN TURBIDITY INCREASE BETWEEN CONTROL AND STANDARD | DIFFERENCE IN TURBIDITY INCREASE BETWEEN CONTROL AND STANDARD, SQUARED |
|---|---|---|---|---|---|---|
| | | 0 HR.† | 4 HR. | | | |
| Control | 0 | 46 | 133 | 87 | | |
| Standard 1 | 1.0 | 47 | 70 | 23 | 64 | 4,104 |
| 2 | 0.5 | 47 | 85 | 38 | 49 | 2,401 |
| 3 | 0.25 | 44 | 102 | 58 | 29 | 841 |
| 4 | 0.125 | 46 | 121 | 75 | 12 | 144 |

*Concentration in saline added to tubes. Final concentration is one-tenth of this.

†This reading corrects for slight differences in optical density of the individual tubes and individual specimens.

tube and each of the standard and unknown tubes is squared and is, by definition, proportional to the amount of antibiotic present. The squared values for the standards are plotted along the abscissa of semilog paper against the known concentrations of aureomycin per milliliter of saline (see Fig. 1 and Table I). A standard curve is drawn through the four points obtained. The concentration of aureomycin in the unknowns is read off this curve using the squared values for the unknowns obtained as described.

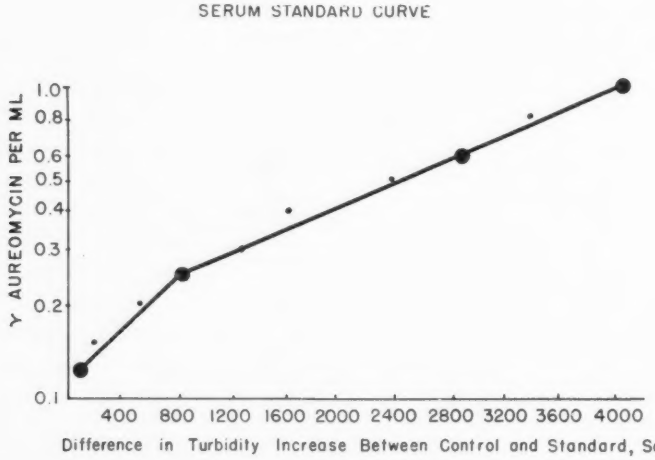


Fig. 1.—The four standard points are plotted in large dots, while twelve simultaneously determined intermediate values are plotted in small dots. The concentrations of aureomycin plotted on the ordinate of this graph are ten times the final concentration of aureomycin in the standard tubes and therefore correspond to the concentration of aureomycin in an unknown solution before it is diluted by addition of saline and standardized test culture (see Table I).

Blood is collected from a finger or heel puncture and the serum is separated for assay as previously described by one of us (A.D.H.).[4] The collecting tube is made either from a three-inch length of glass tubing, with one end sealed, or from a 7.5 by 95 mm. shell vial. The open end is drawn out to a capillary tip and sealed. The wide portion of the tube is held over the flame and an air vent is quickly produced in the softened glass by the expanding air within the sealed tube. For the collection of blood, the capillary tip is broken off near its base. The finger or heel is punctured so that a free flow of blood is obtained. The tube is held in as nearly vertical a position as possible and the blood readily flows into the tube and down to the base. The tubes are stood upright until the blood is clotted; then the tube is filed off just above the surface of the blood. The clot is broken up with an applicator stick and the tube is centrifuged at 1,500 to 2,000 revolutions per minute for fifteen minutes. The supernatant serum is then pipetted off with a capillary pipette for assay. After a little practice with this technique, one can consistently collect 1.5 ml. or more of capillary blood from a single skin puncture.

Unknown serum and spinal fluid specimens are run in 1:1 and 1:8 dilutions,* thus bringing a maximum of 8 $\mu$g per milliliter of aureomycin within the detectable range of the method. Similarly, unknown urine specimens are run in 1:20 and 1:160 dilutions to detect a range of 5 to 320 $\mu$g per milliliter of aureomycin. These predilutions are made in the type of body fluid being assayed. Serum† for them is obtained from the blood bank,

*For maximum accuracy, a separate pipette is used for each step in the dilution of serum specimens.

†Twenty individual specimens of human serum were tested simultaneously under the conditions of this method and no significant difference was found in the growth of the test organism among these sera. Serum, however, does stimulate the growth of the test organism markedly when compared with the assay broth. Keeping the serum concentration constant in all the tubes of a serum assay controls this factor. However, the concentration of aureomycin in an unknown specimen is calculated from the tube with the greatest predilution when there is a choice, since most of the body fluid in prediluted specimens comes from the same source as the body fluid used in the standard and control tubes.

spinal fluid from air encephalographic studies, and urine from laboratory personnel. All body fluid for dilution of unknown specimens is kept frozen until used. Correction for such dilutions is made in calculating the results.

Aureomycin in a concentration of 1 μg per milliliter in serum and 40 μg per milliliter in acid urine shows no appreciable loss of activity when kept at −20° C. for one week. However, aureomycin in spinal fluid in a concentration of 0.25 μg per milliliter shows about a 50 per cent loss of activity if kept at −20° C. overnight. Therefore, unknown specimens of serum and acid urine are kept at −20° C. until a convenient time for assay, but spinal fluid unknown specimens are assayed on the day collected. The pH of all urine used is adjusted to 7.2 just before assay.

Replicate determinations of either controls, standards, or unknowns do not appreciably enhance the accuracy of the method. A separate standard curve is required for each type of body fluid on each day of assay.

## RESULTS

The results of one hundred and four consecutive determinations of known concentrations of aureomycin intermediately spaced between the values used to make up the standard curve are tabulated in Tables III, IV, and V. One experiment using serum is illustrated in Fig. 1 in which it will be noticed that the standard curve approaches a straight line on semilog paper. A similar straight line is obtained with the other two body fluids tested. The percentage error of each determination has been computed. For urine, the range of error is 0 to 42 per cent, with a mean of 12.4 per cent; for spinal fluid, the range is 0 to 35 per cent, with a mean of 12.3 per cent; for serum, the range

TABLE III. RECOVERY OF KNOWN CONCENTRATIONS OF AUREOMYCIN IN URINE FROM 36 CONSECUTIVE DETERMINATIONS

| KNOWN γ AUREOMYCIN PER ML. | | 1.60 | 1.30 | 0.80 | 0.60 | 0.40 | 0.30 |
|---|---|---|---|---|---|---|---|
| Recovered γ aureomycin per ml. | | | | | | | |
| Determination | 1 | 1.48 | 1.20 | 0.84 | 0.56 | 0.36 | 0.26 |
| | 2 | 1.48 | 1.33 | 0.84 | 0.60 | 0.39 | 0.27 |
| | 3 | 1.50 | 1.33 | 0.86 | 0.62 | 0.50 | 0.33 |
| | 4 | 1.55 | 1.40 | 0.90 | 0.62 | 0.50 | 0.36 |
| | 5 | 1.55 | 1.40 | 1.00 | 0.73 | 0.50 | 0.38 |
| | 6 | 1.75 | 1.55 | 1.00 | 0.73 | 0.50 | 0.48 |

TABLE IV. RECOVERY OF KNOWN CONCENTRATIONS OF AUREOMYCIN IN SERUM FROM 36 CONSECUTIVE DETERMINATIONS

| KNOWN γ AUREOMYCIN PER ML. | | 0.80 | 0.60 | 0.40 | 0.30 | 0.20 | 0.15 |
|---|---|---|---|---|---|---|---|
| Recovered γ aureomycin per ml. | | | | | | | |
| Determination | 1 | 0.60 | 0.56 | 0.35 | 0.26 | 0.14 | 0.13 |
| | 2 | 0.74 | 0.60 | 0.36 | 0.28 | 0.16 | 0.13 |
| | 3 | 0.77 | 0.60 | 0.39 | 0.29 | 0.18 | 0.14 |
| | 4 | 0.78 | 0.62 | 0.41 | 0.31 | 0.19 | 0.14 |
| | 5 | 0.78 | 0.62 | 0.42 | 0.31 | 0.20 | 0.14 |
| | 6 | 0.92 | 0.71 | 0.45 | 0.35 | 0.20 | 0.15 |

TABLE V. RECOVERY OF KNOWN CONCENTRATIONS OF AUREOMYCIN IN SPINAL FLUID FROM 32 CONSECUTIVE DETERMINATIONS

| KNOWN γ AUREOMYCIN PER ML. | | 0.87 | 0.80 | 0.75 | 0.62 | 0.60 | 0.40 | 0.37 | 0.30 | 0.20 | 0.15 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Recovered γ aureomycin per ml. | | | | | | | | | | | |
| Determination | 1 | 0.67 | 0.73 | 0.47 | 0.53 | 0.57 | 0.33 | 0.29 | 0.25 | 0.17 | 0.13 |
| | 2 | 1.00 | 0.78 | 0.47 | 0.53 | 0.62 | 0.33 | 0.29 | 0.26 | 0.19 | 0.13 |
| | 3 | – | 0.78 | – | – | 0.62 | 0.40 | – | 0.30 | 0.20 | 0.14 |
| | 4 | – | 0.78 | – | – | 0.68 | 0.44 | – | 0.32 | 0.20 | 0.14 |

is 0 to 35 per cent, with a mean of 8.3 per cent. The accuracy of this method appears to be distinctly superior to that we achieved, in the presence of body fluids, with the original turbidimetric method of Meads and a serial dilution method of aureomycin assay.[3] Furthermore, it is possible to detect 0.1 μg of aureomycin per milliliter or less in the presence of the three body fluids tested, so that its sensitivity compares favorably with the sensitivity of the other methods currently in use.[3] The method is not as accurate in concentrations below 0.125 μg per milliliter as in higher concentrations, however.

## CONCLUSION

A modification of Meads' turbidimetric method of aureomycin assay has been described. It appears to provide a simple and accurate means of assaying low concentrations of aureomycin in small volumes of various body fluids.

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# A MICROMETHOD FOR BLOOD PENICILLIN ASSAY

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IN MAKING frequent penicillin plasma determinations in children, it is helpful if a method can be used which requires such a small amount of blood for each assay that it can be obtained from a puncture wound of the finger as opposed to withdrawal by venipuncture. Fielding[1] described a micromethod requiring as little as 0.25 ml. of fluid for assay in which the test organism was the Oxford strain of *Staphylococcus aureus* grown in glucose serum medium containing Andrade's indicator. The principle of the test was the detection, by means of the color indicator, of the pH change induced in the medium by the breakdown of glucose during the growth of the test organism. When the growth of the organism was prevented by a given amount of penicillin, then no color change would occur. The minimum amount of penicillin which prevented the growth of the organism was that present in the last tube showing no color change.

In the procedure described by Fielding, the test organism and body fluid to be tested were mixed on a paraffined slide, and then taken up in capillary tubes following the technique of Fleming.[2] Because this technique is not commonly employed routinely in this country, it seemed advisable to attempt to utilize the same principle and devise a micromethod employing the more conventional procedures involved in serial test tube dilutions. The method to be described fulfills the following criteria: (1) It requires only that amount of blood which can be taken easily from a finger prick. (2) It uses an easily prepared test medium. (3) The end point is easily read by means of a sharp color change. (4) The procedure is one commonly employed in serology. (5) Finally, its accuracy appears to be superior to that obtained by the widely employed method described originally by Rammelkamp[3] and frequently modified.

## TECHNIQUE

*Collecting of Blood.*—The tube which was found convenient for drawing blood and separating the serum has been described previously[4] and is illustrated in Fig. 1.*

Blood was taken from finger or heel puncture by capillary attraction and allowed to clot in the body of the tube. The tube was then filed and broken off above the level of the blood which was then centrifuged at 1,500 to 2,000 r.p.m. for ten to twenty minutes. The serum that separated was pipetted off for testing.

*Organism.*—The organism used in this laboratory was *Staph. aureus* (F.D.A. strain 209). There is no reason why any penicillin-sensitive, easily cultivated organism could not

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The work described in this paper was done under a contract with the Biological Department, Chemical Corps, MV Division, Camp Detrick, Frederick, Md.

Received for publication, Aug. 1, 1949.

*Shell vials 95 by 7.5 mm. or glass tubing of 7.5 mm. bore and of convenient length (90 to 100 mm.) sealed at one end can be used. The open end is drawn out to capillary tip and sealed. A small hole is blown through the wall of the tube by heating it 25 mm. below the beginning of the capillary portion.

be used equally well. The organism, after a preliminary incubation at 37° C., was kept as a stock culture on plain nutrient agar (Difco) in a refrigerator. It was subcultured once a week onto fresh agar. When required for the test, the organism was transferred to nutrient broth (Difco) and incubated at 37° C. for eighteen hours. A portion was then diluted 1:1,000 in the assay medium to be described below.

Fig. 1.

*Medium.*—The assay medium was phenol red dextrose broth made up as follows:

| | |
|---|---|
| Nutrient broth (Difco) | 8 Gm. |
| Dextrose | 5 Gm. |
| Aqueous phenol red | 25 ml. |
| Distilled water | 1,000 ml. |

This was adjusted to pH 8 with N sodium hydroxide using the phenol red (0.04 per cent concentration) as indicator, the final color being red. After autoclaving for fifteen minutes at 15 lb., the medium was ready to use without further adjustment of the pH.

*Procedure.*—The test was arranged so that one-half step dilutions were used, and a triplicate penicillin control test was included with each series of unknowns.

*Control.*—This was included routinely to detect the sensitivity of the test organism. A penicillin solution was made up in the assay broth in such a way that the penicillin concentration was 0.5 unit per milliliter. The one-half step dilutions were made as follows. Three sets, each containing thirteen plugged, sterile Wassermann tubes (100 by 13 mm.), were arranged in suitable racks. In each set of tubes, the following steps were carried out. The test medium (phenol red dextrose broth) was added in 0.1 ml. amounts to tubes 1, 3, and 4 to 12 inclusive. Tube 2 was left empty, and 0.2 ml. of the test medium was added to tube 13. The penicillin solution was then added in 0.1 ml. amounts to tube 1 which made a 1:2 dilution and to tube 13 which made a 1:3 dilution. Tube 1 (1:2 dilution) was then used after thorough mixing, for preparing one series of twofold (one step) dilutions by transferring 0.1 ml., serially to the odd numbered tubes 3 to 11, 0.1 ml. from tube 11 being discarded. From tube 13, which contained 1:3 dilution of penicillin, 0.1 ml. was added to the empty tube 2 (1:3 dilution) and to tube 4 which already contained 0.1 ml. assay broth (1:6 dilution). Tube 13 was then discarded. Tube 4, after thorough mixing, was used to initiate the second series of twofold (one step) dilutions by transferring 0.1 ml. serially to the even numbered tubes 6 to 12, 0.1 ml. from tube 12 being discarded. Tubes 1 to 12 in each set then contain 0.1 ml. of assay medium, with concentrations of penicillin decreasing from 1:2 to 1:96 by one-half steps.

*Test Fluids.*—A maximum of 0.2 ml. was required for each test fluid. Except that only one set of tubes was run, this was handled in a similar manner to that just described, the fluid taking the place of the known penicillin solution.

*Test Organism.*—0.1 ml. of the 1:1,000 dilution of the eighteen-hour culture of the test organism described was then added to every tube and the complete series incubated overnight at 37° C.

*End Point.*—Since, with this indicator, a change from red to yellow indicates a lowering of pH resulting from bacterial growth, the end point was taken as the last tube in each set in which the original red color of the medium was unaltered. This end point was usually quite sharp. Since the controls were set up in triplicate, it sometimes occurred that the end point varied somewhat in one of the three sets, although this variation was seldom more than one-half step off in either direction. The end point taken was that reached in two of the three control sets. If there was no such agreement, the test was considered invalid.

*Calculation.*—First, the sensitivity of the organism was calculated from the controls. Since the concentration of penicillin in the first tube of each control series was 0.5 unit per milliliter, the concentration in any subsequent tube could be calculated by dividing 0.5 unit per milliliter by the reciprocal of the dilution of that tube. For example, if the end point in two of the three control series was found to be tube 7 or a 1:16 dilution, then the organism was sensitive to a concentration of $\frac{0.5}{16}$ or 0.03 unit per milliliter.

The concentration of penicillin in any unknown body fluid was easily calculated by multiplying the sensitivity of the organism, as determined in the preceding paragraph, by the reciprocal of the dilution of body fluid contained in the end point tube. For example, if in the test the sensitivity of the organism was 0.03 unit per milliliter and the end point of the body fluid being tested was found to be tube 5 or 1:8 dilution, then the concentration of penicillin in the original body fluid was 0.03 × 8 or 0.24 unit per milliter.

In the laboratory it was found convenient to record the dilution and the end points of the controls and the test fluids on squared paper. This avoided any mistake in the dilutions (Table I).

TABLE I. SAMPLE CHART FOR MICRO-PENICILLIN ASSAY

| TUBE | | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| DILUTION | | 2 | 3 | 4 | 6 | 8 | 12 | 16 | 24 | 32 | 48 | 64 | 96 | PENICILLIN (UNITS/ML.) DETECTED |
| PENICILLIN (UNITS/ML.) | | 0.25 | 0.17 | 0.125 | 0.085 | 0.065 | 0.04 | 0.03 | 0.02 | 0.015 | 0.01 | 0.0075 | 0.005 | |
| Control | 1 | | | | | | | x | | | | | | 0.03 |
| | 2 | | | | | | | x | | | | | | |
| | 3 | | | | | | x | | | | | | | |
| Pt. 1 | ½ hr. | | | | | | | | | x | | | | 0.96 |
| | 1½ hr. | | | | | | x | | | | | | | 0.36 |
| | 3 hr. | | | x | | | | | | | | | | 0.12 |
| Pt. 2 | ½ hr. | | | | | | | | | | x | | | 1.44 |
| | 1½ hr. | | | | | | | x | | | | | | 0.48 |
| | 3 hr. | | | | x | | | | | | | | | 0.18 |
| Pt. 3 | ½ hr. | | | | | | | x | | | | | | 0.48 |
| | 1½ hr. | | | | | | | x | | | | | | 0.48 |
| | 3 hr. | | | | | x | | | | | | | | 0.24 |

*Accuracy of the Method.*—This method was first tested on five different occasions by attempting on each occasion to determine the amount of penicillin in each of five twofold serial dilutions of a known concentration of penicillin in distilled water. Table II shows the results obtained by the method just described. It can be seen that the recoveries at each dilution were remarkably close to the amount of penicillin actually present. The average of the tests was ± 8 per cent, although individual readings sometimes deviated as much as 44 per cent. Comparison with the results obtained by the Rammelkamp method

TABLE II. DETERMINATION OF ACCURACY OF MICROMETHOD OF PENICILLIN ASSAY

| KNOWN PENICILLIN CONCENTRATION | UNITS/ML. OF PENICILLIN IN SERUM | | | | |
|---|---|---|---|---|---|
| | 0.1 | 0.5 | 1.0 | 2.0 | 4.0 |
| Test 1 | 0.12 | 0.48 | 1.08 | 1.92 | 3.84 |
| Test 2 | 0.12 | 0.72 | 1.08 | 1.92 | 3.84 |
| Test 3 | 0.12 | 0.48 | 1.08 | 1.92 | 3.84 |
| Test 4 | 0.12 | 0.72 | 0.72 | 1.92 | 3.84 |
| Test 5 | 0.09 | 0.48 | 1.08 | 1.92 | 3.84 |
| Average | 0.11 | 0.57 | 1.0 | 1.92 | 3.84 |

(Table III) indicates that the micromethod was more accurate. However, there appeared to be a definite relationship between the two, the micromethod giving a consistently higher reading by a factor of 1.6. Fig. 2 shows a graphic comparison of the known concentrations of penicillin and the recoveries by the two methods used.

TABLE III. DETERMINATION OF ACCURACY OF THE MODIFIED RAMMELKAMP METHOD OF PENICILLIN ASSAY

| KNOWN PENICILLIN CONCENTRATION | UNITS/ML. OF PENICILLIN IN SERUM | | | | |
|---|---|---|---|---|---|
| | 0.1 | 0.5 | 1.0 | 2.0 | 4.0 |
| Test 1 | 0.052 | 0.3 | 0.832 | 1.22 | 2.43 |
| Test 2 | 0.052 | 0.3 | 0.832 | 1.22 | 2.43 |
| Test 3 | 0.052 | 0.3 | 0.624 | 1.22 | 2.43 |
| Test 4 | 0.052 | 0.3 | 0.624 | 1.22 | 2.43 |
| Test 5 | 0.052 | 0.3 | 0.624 | 0.91 | 2.43 |
| Average | 0.052 | 0.3 | 0.71 | 1.15 | 2.43 |

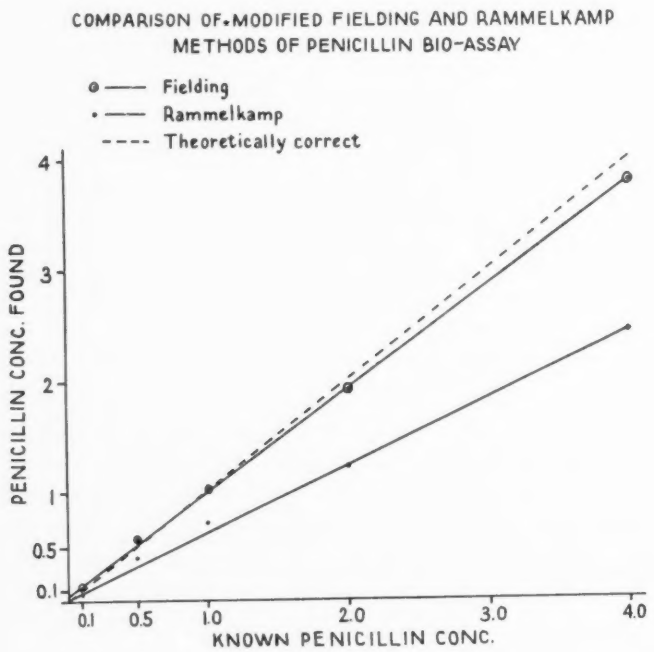


Fig. 2.

Following the demonstration that known amounts could be assayed with a good degree of accuracy, this test has been used successfully for hundreds of routine assays of penicillin levels in body fluids.

## SUMMARY

A micromethod for the estimation of penicillin levels in blood or other body fluids, requiring only 0.2 ml. for the test, is described. Sufficient blood can be collected conveniently from a single puncture wound of the finger. A convenient collecting tube for obtaining serum is described and illustrated.

The principle employed in the test is the detection of growth of the test organism in a glucose-containing medium by the demonstration of acid formation by the color change of a suitable indicator. The end point is taken as the last tube to show no evidence of color change. By including controls in each test, the quantity of penicillin in an unknown penicillin fluid can be calculated. The accuracy of the method is greater in our hands than the commonly employed Rammelkamp method, and it has proved a convenient assay method for routine use in children.

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# THE PRODUCTION OF ANTIRABBIT HEMOLYSIN

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THE hemolytic system most commonly used in complement fixation tests consists of sheep erythrocytes and antisheep hemolysin. A supply of fresh sheep erythrocytes for the performance of the test necessitates constant maintenance of a few sheep on the premises, which is often inconvenient. Attempts have been made by Vedder[1] and by me[2] to overcome this difficulty by a different hemolytic system. The object of the present communication is to describe another modification, in which the role of rabbit and sheep is reversed. Rabbit erythrocytes are employed with an antirabbit hemolysin of the sheep.

## METHODS AND MATERIALS

Five sheep were selected for this study, two adult males, two adult females, and one female lamb.

To each 50 ml. of freshly drawn rabbit blood about 2 ml. of 0.4 per cent sodium citrate were added to prevent clotting. The erythrocytes were washed three times in sterile 0.9 per cent NaCl solution and the packed red blood corpuscles were resuspended in equal volume of saline for inoculum.

Hemolyzed rabbit red blood corpuscles and the stroma of rabbit erythrocytes as prepared by Vedder were tried out as an alternate inoculum.

The inoculations were either intravenous or subcutaneous or both, and the dose for each inoculum varied from 5 ml. to 200 milliliters. Control blood samples were taken from the sheep before initial inoculations. Samples were taken also at the end of each course of immunization and after inactivation at 56° C. for thirty minutes they were tested for hemolysin.

Antirabbit hemolysin titrations were made with 2 per cent washed rabbit erythrocytes. In some of the tests, titrations were made with blood taken from the same rabbits that had been bled previously for immunization of the sheep.

Pooled guinea pig complement in dilutions of 1:15 and 1:30 was used in the hemolysin titrations. The titration was checked by using the standard Kolmer hemolysin titration test with antisheep hemolysin and 2 per cent sheep red corpuscles.

Wassermann-positive and Wassermann-negative human sera were tested with the antirabbit hemolysin and 2 per cent washed rabbit erythrocytes by the modified Kolmer method and with the standard Kolmer-Wassermann complement fixation test, Kolmer,[3] and the results were compared.

## EXPERIMENTAL DATA

The five sheep used in this study differed in their production of antirabbit hemolysin.

The sheep which were inoculated with large doses of rabbit erythrocytes at frequent intervals produced hemolysin promptly and in higher titer as compared with the sheep which were inoculated with smaller doses of erythrocytes,

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This study was supported by the United States Navy, Office of Naval Research.

The writer wishes to express his thanks to Dr. W. B. Sharp, Miss Lorraine Turck, Dr. Frank Wappler, and Dr. Louise Wilcox for their interest and assistance in this study.

Received for publication, Aug. 1, 1949.

even when inoculations were spread over a longer period of time. When sheep were immunized for hemolysin, it was noted that agglutinin for rabbit erythrocytes occurred much sooner and in higher titer than did antirabbit hemolysin, particularly when the period of immunization was prolonged. Thus Sheep A at the end of seventeen days had no hemolysin while the agglutinin titer was 1:2,400. The same sheep at the end of ninety-three days of immunization had a hemolysin titer of 1:30 and the agglutinin titer was 1:10,240.

In Sheep D and E, which were immunized in massive doses and at frequent intervals, agglutinin and hemolysin occurrence were almost simultaneous.

The results of the production of antirabbit hemolysin in sheep are summarized in Table I.

Sheep A (male), which was immunized with small quantities (5 to 10 ml.) intravenously of washed rabbit erythrocytes, produced hemolysin after sixty-nine days of immunization; however, during the entire course of immunization (ninety-three days), hemolysin titer never exceeded 1:30.

Sheep B (male) received sixty-nine injections totaling 250 ml. of lysed rabbit erythrocytes and 2,455 ml. of washed rabbit erythrocytes in a period of 290 days. The immunization of this sheep was begun with small amounts (10 ml.) of hemolyzed red blood corpuscles. During fifty days of immunization, this sheep was inoculated nineteen times with 190 ml. of hemolyzed erythrocytes. At the end of this period, the hemolysin titer was only 1:4. Since the lysed corpuscles failed to produce a significant hemolysin titer and inasmuch as Sheep A already had produced hemolysin following injection of intact erythrocytes, at this stage the inoculation of lysed erythrocytes was discontinued. Subsequent inoculations with rather large doses (25 to 100 ml. per inoculum) of intact erythrocytes increased the hemolysin titer to 1:80. Prolongation of immunization, instead of increasing, decreased the hemolysin titer to 1:20 (see Table I).

Sheep C (female) received thirty injections totaling 1,400 ml. of washed rabbit erythrocytes and 110 ml. of stroma of rabbit erythrocytes during a period of 234 days. The immunization of this sheep was begun with rather large doses of washed rabbit erythrocytes and the highest hemolysin titer obtained was 1:20. Since the sheep manifested mild anaphylactic symptoms, intravenous inoculations were substituted with stroma. In spite of further immunization with stroma, which subsequently was followed with intact erythrocytes, the hemolysin titer did not increase (see Table I).

Sheep D (pregnant female) was immunized with fifty-four injections totaling 4,200 ml. of washed rabbit erythrocytes in a 212-day period. The immunization of this sheep was done with massive doses and at frequent intervals. Hemolysin with titer of 1:40 was present as early as twenty-three days following initial inoculation. While the sheep was undergoing immunization, it gave birth to a lamb. Four days after the lamb was born, blood samples taken from the sheep showed hemolysin titer of 1:320. Subsequent inoculations with 50 and 100 ml. of washed erythrocytes were at times accompanied with slight to moderate anaphylactic reactions such as sneezing, coughing, discharge of feces and bloody urine, weakness, inability to move, salivation, tremors, and dyspnea.

TABLE I. THE PRODUCTION OF ANTIRABBIT HEMOLYSIN IN SHEEP

| SHEEP | INOCULATION | ERYTHROCYTES | | HEMOLYZED ERYTHROCYTES | | STROMA | | ROUTE OF INOCULATION | DAYS BETWEEN EACH INOCULATION | REST PERIOD (DAYS) | SEROLOGIC TESTS | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | ML. PER EACH INOCULATION | TOTAL ML. | ML. PER EACH INOCULATION | TOTAL ML. | ML. PER EACH INOCULATION | TOTAL ML. | | | | DAYS AFTER FIRST INOCULATION | HEMOLYSIN TITER |
| A | 1- 9 | 5 | 45 | – | – | – | – | I.V. | 2 to 3 | 26 | 17 | 0 |
| | 10-18 | 10 | 90 | – | – | – | – | I.V. | 2 to 8 | 3 | 69 | 1:24 |
| | 19-27 | 10 | 90 | – | – | – | – | S.C. | 1 to 5 | 3 | 93 | 1:30 |
| B | 1-10 | – | – | 10 | 100 | – | – | I.V. | 2 to 4 | 3 | 26 | 0 |
| | 11-19 | – | – | 10 | 90 | – | – | S.C. | 2 to 5 | 3 | 50 | 1:4 |
| | 20-22 | – | – | 5-40 | 60 | – | – | I.V. and S.C. | 3 | 4 | --- | --- |
| | 23-29 | 25 | 175 | – | – | – | – | S.C. | 4 to 5 | 5 | 96 | 1:40 |
| | 30-40 | 50 | 550 | – | – | – | – | I.V. | 3 to 6 | 2 | 140 | 1:80 |
| | 41-46 | 50 | 300 | – | – | – | – | I.V. | 1 to 3 | 2 | --- | --- |
| | 47-52 | 50 | 300 | – | – | – | – | S.C. | Daily | 4 | 161 | 1:80 |
| | 53-60 | 40-70 | 380 | – | – | – | – | I.V. | 2 to 3 | 20 | 218 | 1:40 |
| | 61-66 | 100 | 600 | – | – | – | – | I.V. | 2 to 3 | 3 | 239 | 1:20 |
| | 67-69 | 50 | 150 | – | – | – | – | I.V. | 1 to 3 | 26 | 290 | 1:20 |
| C | 1- 7 | 50 | 350 | – | – | – | – | I.V. | 3 to 4 | 2 | 23 | 1:10 |
| | 8-14 | 50-100 | 600 | – | – | – | – | I.V. | 2 to 4 | 146 | 44 | 1:20 |
| | 15-21 | – | – | – | – | 5-8 | 70 | I.V. | 2 to 11 | 4 | --- | --- |
| | 22-30 | 50 | 450 | – | – | 8 | 40 | S.C. and I.V. | 1 to 2 | 30 | 234 | 1:10 |
| D | 1-10 | 50 | 500 | – | – | – | – | I.V. | 2 to 3 | 2 | 23 | 1:40 |
| | 11-18 | 50-100 | 700 | – | – | – | – | I.V. | 2 to 3 | 21 | 44 | 1:320 |
| | 19-25 | 50-100 | 700 | – | – | – | – | I.V. and S.C. | 2 to 3 | 3 | 77 | 1:80 |
| | 26-28 | 200 | 600 | – | – | – | – | I.V. and S.C. | 2 | 24 | 86 | 1:80 |
| | 29-35 | 100 | 700 | – | – | – | – | I.V. | 2 to 3 | 24 | 122 | 1:40 |
| | 36-38 | 50 | 150 | – | – | – | – | I.V. | 1 to 3 | 30 | 173 | 1:80 |
| | 39-50 | 50 | 600 | – | – | – | – | I.V. | 2 to 3 | 4 | 204 | 1:40 |
| | 51-54 | 50-100 | 250 | – | – | – | – | I.V. and S.C. | 1 to 3 | --- | 212 | --- |
| E | 1- 7 | 100-200 | 800 | – | – | – | – | I.V. | 2 to 3 | 24 | 17 | 1:10 |
| | 8-10 | 50 | 150 | – | – | – | – | I.V. | 1 to 3 | 30 | 68 | 1:20 |
| | 11-16 | 50 | 300 | – | – | – | – | I.V. | 2 to 3 | 2 | 85 | 1:10 |
| | 17-22 | 50 | 300 | – | – | – | – | I.V. | 2 to 3 | 4 | 99 | 1:10 |
| | 23-35 | 50-100 | 750 | – | – | – | – | S.C. and I.V. | 1 to 12 | 30 | 137 | 1:20 |
| | 36-40 | 30- 50 | 210 | – | – | – | – | S.C. | 2 to 3 | 12 | 180 | 1:10 |

In spite of continuation of immunization, the hemolysin titer never exceeded 1:320, but diminished to 1:40. During the last intravenous inoculation, the sheep died of anaphylactic shock.

Sheep E. This female lamb, born to immunized Sheep D, was tested at the age of 2 months for the presence of possible antirabbit hemolysin, with negative results. This was followed by forty injections of rabbit red blood corpuscles, totaling 2,510 ml. during 180 days. The first seven inoculations were made intravenously in 200 ml. amounts every other day. The highest hemolysin titer obtained at various stages of immunization was 1:20 (see Table I).

Antirabbit hemolysin with a titer of 1:40 or above and a 2 per cent suspension of washed rabbit erythrocytes was tested with Wassermann-positive and Wassermann-negative human sera by Kolmer technique; because of the low hemolytic titer of antirabbit hemolysin, relatively large amounts of this hemolysin were used. As a control, parallel tests were made with Kolmer-Wassermann method. Forty-two syphilitic and forty-two normal sera were run by each method. The results of this test were clear-cut, and known positive samples gave positive readings and negative samples gave negative readings by both procedures. The findings by the experimental method were identical with those obtained with antisheep hemolysin and washed sheep erythrocytes, as used in the usual Wassermann test.

## SUMMARY

Antirabbit hemolysin was produced in sheep by inoculating them intravenously and subcutaneously with washed rabbit red blood corpuscles. The hemolysin titer acquired by these animals ranged from 1:10 to as high as 1:320.

Hemolyzed erythrocytes and stroma injected into sheep for the production of hemolysin did not produce as high a titer as washed erythrocytes.

Antirabbit hemolysin and 2 per cent washed rabbit erythrocytes were used in testing syphilitic and normal sera by modified Kolmer-Wassermann technique. The results obtained in every instance were clear-cut and of diagnostic value.

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# DIFFERENTIATION AND ENUMERATION OF EOSINOPHILS IN THE COUNTING CHAMBER WITH A GLYCOL STAIN; A VALUABLE TECHNIQUE IN APPRAISING ACTH DOSAGE

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THE decrease in the circulating eosinophils following the intramuscular injection of adrenocorticotrophic hormone (ACTH, Armour) was first described by Hills, Forsham, and Finch[1] and subsequently by Hellman.[2] The increasing importance of ACTH in the diagnosis[3] and treatment[4, 5] of various clinical conditions as well as in other clinical studies of the effects of this hormone[6, 7] underscores the importance of a simple, reliable technique for enumerating eosinophils, as the level of these cells in the peripheral blood may be a general index of the sensitivity of the gland stimulation and may be a general guide to adequacy of dosage in a given case.[8, 9]

In this connection the merits of a white blood cell diluting fluid of equal parts propylene glycol and water containing phloxine and methylene blue as stains should be re-emphasized because it is the simplest and most accurate method of enumerating blood eosinophils. This point is important because the number of circulating eosinophils is a general guide of adequate dosage of ACTH in treating various disease syndromes. Although our own observations in this respect† are not ready for publication, a review of the technique applied in enumerating eosinophils seems indicated because of the rapidly increasing medical interest in adrenocorticotrophic hormone (ACTH).

Hypotonic diluting fluids of the type originally described by Dunger[10] or as modified by Hills, Forsham, and Finch[1] and Thorn and associates[3] are not entirely satisfactory for counting eosinophils in the peripheral blood. This statement is based on the experience of employing Camara and Alvarez'[11] modification of Dunger's diluting fluid consisting of 5 parts of 1 per cent aqueous eosin, 5 parts acetone, and 100 parts distilled water for a period of three years in studying cases of food and drug allergy. The staining fluid used by those studying the effects of ACTH is identical except that 2 per cent instead of 1 per cent aqueous eosin was employed.

The inadequacies of this type of diluting fluid were first pointed out in 1944.[12] It was determined that the counting chamber estimation of eosinophils by means of hypotonic diluting fluids containing eosin as modified from Dunger's original description remains subject to considerable error in that the ruptured or "ghost" cells are identified by clumps of eosin-staining granules retained in portions of the cell membrane. In 1947 it was again emphasized by me[13] that the use of such diluents was fraught with considerable error in that one frequently encountered indeterminate forms that could not with certainty

Received for publication, Aug. 19, 1949.

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†These studies were conducted through the cooperation of Dr. David E. Markson and Dr. Smith Freeman who are studying the effect of ACTH on disease syndromes. The ACTH was obtained through the courtesy of Dr. John R. Mote of the Armour Laboratories.

be classified as eosinophils. In addition, a greater absolute number of eosinophils usually was obtained from employing this type of hypotonic diluent than was determined from performing parallel stained film differential counts. It was further pointed out, however, that the errors inherent in this method became less significant if the determinations were made with a short but constant time interval elapsing between the dilution of the sample and the completion of the counting. These observations have recently been confirmed by Henneman, Wexler, and Westenhaver.[14] In their experience with the Dunger technique as modified by Thorn and associates, there was a large and rapid decrease in the eosinophil cell count with passage of time after dilution of the blood and with ageing of the oxalated blood. Because of this and other reasons, these authors were unable to recommend the use of this type of diluent.

The so-called "hemolytic" and solvent properties of propylene glycol suggested its use as a white cell diluting fluid and staining base in 1943.[15] Upon closer observation it was learned that the apparent hemolytic action of the glycols, as reported by Von Oettingen and Jirouch,[16] was not a true hemolysis, but that the disappearance of human erythrocytes suspended in equal parts propylene glycol and water was due to the fact that the red blood cells rapidly assumed the same density as the surrounding media and thus became relatively nonrefractile. This phenomenon was studied in more detail by Randolph and Mallery[17] who were able to demonstrate that a mixture of human blood in equal parts propylene glycol and water appearing to be hemolyzed as viewed grossly in a test tube or under the light microscopic field actually was not hemolyzed when viewed through the dark-field microscope. Under these circumstances the erythrocytes retained their normal contours.

The technique described in 1944[12] and employed in subsequent studies consists of the following:

Given amounts of stock solutions containing 0.1 per cent methylene blue in propylene glycol and 0.1 per cent phloxine in propylene glycol are each diluted with an equal volume of distilled water and placed in dropper bottles. For example:

| Solution I* | |
|---|---|
| 0.1 per cent methylene blue in propylene glycol | 50.0 c.c. |
| Distilled water | 50.0 c.c. |
| **Solution II*** | |
| 0.1 per cent phloxine in propylene glycol | 50.0 c.c. |
| Distilled water | 50.0 c.c. |

The final white blood cell diluting fluid is made by mixing an equal number of drops of Solution I and Solution II in a test tube; this remains usable for approximately four hours. After standing for longer periods, differential staining detail may become impaired and precipitation of dyes may occur. Curiously, prior to four hours the two dyes do not seem to mix completely as shown by the ability of the phloxine to disperse in a filter paper at a greater rate of speed than does the methylene blue, a drop on such a medium separating as to color and leaving a blue center and a red periphery in a manner akin to the principle employed in paper chromatography.

*As has been pointed out,[17] the effect of propylene glycol on erythrocytes as far as the timing of the "reappearance" phase is concerned is dependent upon concentration of the glycol and the temperature. Occasionally the loss of water from the stock or working solutions due to evaporation increases the relative concentration of propylene glycol to the point that in summer weather the red cells may "reappear" before one has an opportunity to enumerate the leucocytes. This is remedied by making up solutions containing 40 or 45 per cent propylene glycol instead of the recommended 50 per cent.

Because of the variation in staining ability between lots of dyes, optimum acid and basic staining may sometimes be obtained by making a slight change in the relative proportions of Solution I and II in the final diluent. For example, four parts of Solution I and six parts of Solution II might result in better staining technique than if equal parts of the two were mixed.

Under certain circumstances the phloxine tends to crystallize out in long narrow crystals from Solution II; this may occur after the solution has stood several weeks and may be remedied readily by simple filtration or by preparing Solution II from the stock at monthly intervals.

Experience indicates that it is helpful to clean the pipettes with agents other than acids or to be certain that acid radicles are completely removed from the interiors of the pipettes as a result of repeated rinsing. Cleaning the pipettes with water and drying by a final rinse with acetone and avoiding the use of a series of bottles also employed in cleaning pipettes containing acid diluents aids in avoiding complications in the use of this technique.

Total and differential white blood cell counts are made using the standard pipette and counting chamber. The pipette is shaken during the course of and immediately after filling. If the chamber is charged immediately after diluting the blood and shaking, a period of from three to five minutes is required for the "disappearance" of the red blood cells and settling of the leucocytes prior to determining the total white cell count. A total period of from ten to fifteen minutes is recommended for maximum staining of eosinophils and other leucocytes. Blood may be left in the pipette for varying periods, including overnight, without a significant change in the staining qualities of the cells or in the total leucocyte count, an obvious advantage over the use of hypotonic diluents employed in enumerating eosinophils.

Under the low magnification (16 mm. objective) eosinophils may be differentiated from other cells by their brilliant red color and slightly larger size. The contrast in color is intensified by increasing the light and raising the condenser of the microscope. The eosinophils in one side of the counting chamber (0.9 c.mm.) or in the entire ruled areas of both sides of the chamber (1.8 c.mm.) are counted and multiplied, respectively, by 22 and 11 to obtain the number per cubic millimeter of blood.

The differentiation and enumeration of polymorphonuclear and mononuclear cells are accomplished to best advantage by the use of high magnification (4 mm. objective and 15× eyepiece). The first 100 cells encountered are so classified.

In this diluting fluid all white blood cells remain intact, round in contour, and relatively large in size. The granules of the eosinophils stain brilliant red, the nuclei of all cells aquamarine; this permits the differentiation of eosinophils from other white blood cells even in the presence of highly fragile leucocytes as observed in leucemia and other blood disorders.[18]

The increased viscosity of this medium is advantageous in that the end point in filling the pipette may be reached accurately, there is little settling and clumping of white cells, and there is no tendency toward overflow in charging the chamber. The clear background of the counting chamber and the relatively slow rate of evaporation from it are also advantageous.

The differentiation of eosinophils in the same sample of blood employed in determining the total leucocyte count results in a minimum possible error in comparison with alternative methods.

Finally, the glycol-stain technique also permits the counting chamber enumeration of polymorphonuclear and mononuclear leucocytes even in the presence of many young myeloid elements as observed in the course of acute allergic reactions following the ingestion of allergenic drugs.[19]

The accuracy of the counting chamber enumeration of eosinophilis on one side of the chamber (0.9 c.mm.) using the propylene glycol technique as compared with the standard method of enumerating eosinophils, that is, by determining their number as a result of performing 200 cell film differential counts and applying the percentage obtained to the total leucocyte count, was studied by Randolph and Stanton,[20] employing parallel observations from the same freely bleeding puncture wound. Such determinations were made in 100 normal persons and in 400 instances in allergic individuals who had varying degrees of eosinophilia. Finally, ten of the latter subjects were subjected to ten parallel eosinophil counts each by the two techniques.

Table I lists the maximum, minimum, mean, and standard deviation determined as a result of the statistical analysis of these ten observations per subject by each of the two techniques.

TABLE I. EOSINOPHIL COUNTS AS DETERMINED IN ENUMERATING THE EOSINOPHILS IN ONE SIDE OF THE COUNTING CHAMBER (0.9 C.MM.) COMPARED WITH THE DIFFERENTIAL ENUMERATION OF EOSINOPHILS FROM PERFORMING 200 CELL DIFFERENTIAL COUNTS FROM THE STAINED FILM

| | EOSINOPHILS IN 0.9 C.MM. OF CHAMBER WITH THE PROPYLENE GLYCOL TECHNIQUE | | | | EOSINOPHILS FROM 200 CELL FILM DIFFERENTIAL COUNTS | | | |
|---|---|---|---|---|---|---|---|---|
| SUBJECT | MAX. | MIN. | MEAN | S. D. | MAX. | MIN. | MEAN | S. D. |
| Ch. | 110 | 44 | 68 | 27.6 | 113 | 0 | 61 | 46.0 |
| H. | 710 | 422 | 562 | 83.4 | 722 | 420 | 553 | 100.0 |
| G. | 799 | 555 | 673 | 92.0 | 1,241 | 453 | 707 | 222.2 |
| F. | 799 | 666 | 726 | 53.6 | 1,278 | 402 | 676 | 229.4 |
| M. | 977 | 888 | 932 | 39.8 | 1,101 | 591 | 785 | 153.0 |
| Q. | 1,088 | 755 | 951 | 117.3 | 1,155 | 532 | 849 | 204.0 |
| Co. | 1,379 | 755 | 1,097 | 203.0 | 1,411 | 981 | 1,204 | 135.5 |
| A. | 2,065 | 1,121 | 1,461 | 303.2 | 1,937 | 1,061 | 1,448 | 249.2 |
| E. | 1,709 | 1,332 | 1,474 | 158.2 | 1,725 | 1,003 | 1,397 | 234.5 |
| B. | 1,865 | 1,221 | 1,543 | 197.3 | 2,218 | 1,484 | 1,850 | 216.0 |

From these studies it was determined that the enumeration of eosinophils on one side of the counting chamber (0.9 c.mm.) was a more accurate method of determining the number of these cells than the result of a differential count of 200 leucocytes from the stained film, employing the cover slip technique and a standard method of sampling the stained area.

Henneman, Wexler, and Westenhaver[14] recently compared the effectiveness of phloxine in equal parts propylene glycol in water (Solution II of this combined stain) with Thorn's modification of Dunger's hypotonic diluting fluid in enumerating eosinophils. In their experience with these two chamber techniques, the diluent composed of phloxine in propylene glycol not only provided more consistent results and no evidence of destruction of eosinophils but

also was preferred by them because of the ease in identification of cells, decreased evaporation of the diluent from the counting chamber, greater reproducibility of results, and because no refrigeration of the diluent was required. They confirmed our experience in using the eosin-acetone hypotonic diluent, commenting specifically on the rupturing of eosinophils in this medium and the large and rapid decrease in cell count with passage of time after dilution of the blood and with ageing of the oxalated blood.

Because of the more accurate enumeration of eosinophils permitted with the use of the glycol counting chamber stains, either the phloxine part of the diluent alone or the combined stain diluting fluid is preferred over alternative methods in following the levels of blood eosinophils in patients under treatment with adrenocorticotrophic hormone (ACTH).

## SUMMARY

A white blood cell diluting fluid of phloxine and methylene blue dissolved in equal parts propylene glycol and water permits the counting chamber differentiation of eosinophils on the same blood specimen used in determining the total leucocyte count. In this medium the eosinophils stain bright red and are readily differentiated in the counting chamber from other polymorphonuclear and mononucleated leucocytes. This technique is the most accurate method of determining the number of eosinophils in the peripheral blood in that it permits the enumeration of intact stained cells in contrast to the previously described counting chamber techniques employing hypotonic diluents in which ruptured or "ghost" eosinophils are counted. It is a particularly valuable method to use in following the clinical course of patients under therapy with adrenocorticotrophic hormone (ACTH), those suffering from acute allergic reactions or infections in which one is primarily interested in the variations in serial determinations of the peripheral blood eosinophils.

This direct counting chamber technique makes it possible for the first time to express accurately the number of eosinophils existing in the peripheral blood; the expression of the number of eosinophils per cubic millimeter of blood is obviously more accurate and meaningful than to consider eosinophils in terms of the relative percentage of the total leucocyte count.

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# WARBURG MANOMETER CALIBRATOR*

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## INTRODUCTION

IN CALCULATING the constant for a Warburg manometer it is necessary to know the volume of the Warburg vessel plus the volume of the manometer to the 150 mm. mark. To determine the latter volume the manometer is usually filled with mercury to the 15 cm. mark and the volume is determined from the weight and density of mercury. This method of calibration is very time consuming since it is somewhat difficult to introduce the requisite amount of mercury to just fill the manometer to the 15 cm. mark. The manometer calibrator herein described greatly simplifies and shortens the calibration procedure. The principle employed is similar to that used in the construction of the Scholander microburette.†

## CONSTRUCTION OF CALIBRATOR

The apparatus consists of a metric micrometer (Fig. 1, *A*) which is calibrated in hundredths of a millimeter. A stainless steel plunger (*B*) with a cross sectional area equal to 1 sq. cm. (1.000) is pressed onto the micrometer spindle (*C*). A stainless steel collar (*D*) is pressed over the end of the micrometer. The upper end of this collar is threaded to accommodate the Lucite top (*E*). This plastic top is turned to a standard taper joint at *H* so that it fits the conventional Warburg manometer. A doughnut shaped "O" ring (*F*) fits over the plunger (*B*) and is compressed between the plastic (*E*) and the collar (*D*). This "O" ring seals the plunger within the plastic, yet it permits advancement of the plunger into the space (*G*).

## METHOD OF CALIBRATION

In use, the plastic cylinder is filled with water and the spindle is nearly withdrawn from the chamber (*G*). The cleaned manometer is inserted in the standard taper (*H*) of the plastic and fixed in place with rubber bands (Fig. 2). It is desirable to have just sufficient water in the calibrator chamber (*G*) so that only a small air bubble remains within the plastic cylinder when the manometer is in place. The manometer is held in the vertical position and the stopcock (*M*) of the manometer is opened and the other ends of the monometer are sealed by rubber tubes (*N*) and pinch clamps (*O*). The micrometer plunger is advanced and all the air is carefully expelled from the calibration chamber (*G*). The water meniscus is adjusted to coincide with the calibration mark of the manometer (*J*) (Fig. 2) (i.e. the mark used in calibrating the vessels) and the micrometer scale is read. The manometer is now turned to a horizontal position

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Received for publication, Aug. 9, 1949.

*Manufactured by Micrometric Instrument Company, 7929 Kinsman Ave., Cleveland 4, Ohio.

†Scholander, P. F., Edwards, G. A., and Irvine, L.: Improved Micrometer Burette, J. Biol. Chem. **148**: 495, 1943.

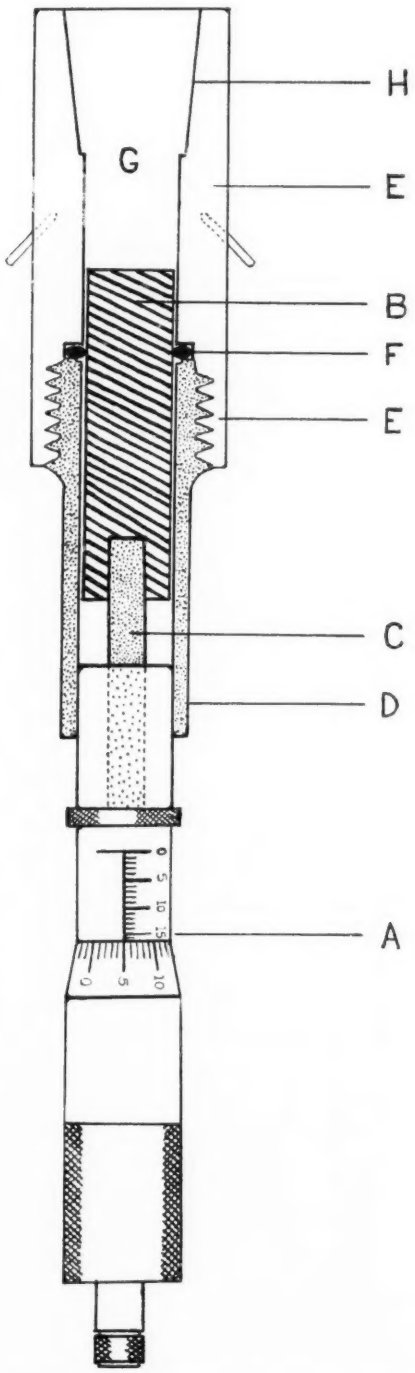


Fig. 1.—Construction of Warburg manometer calibrator (for explanation see text).

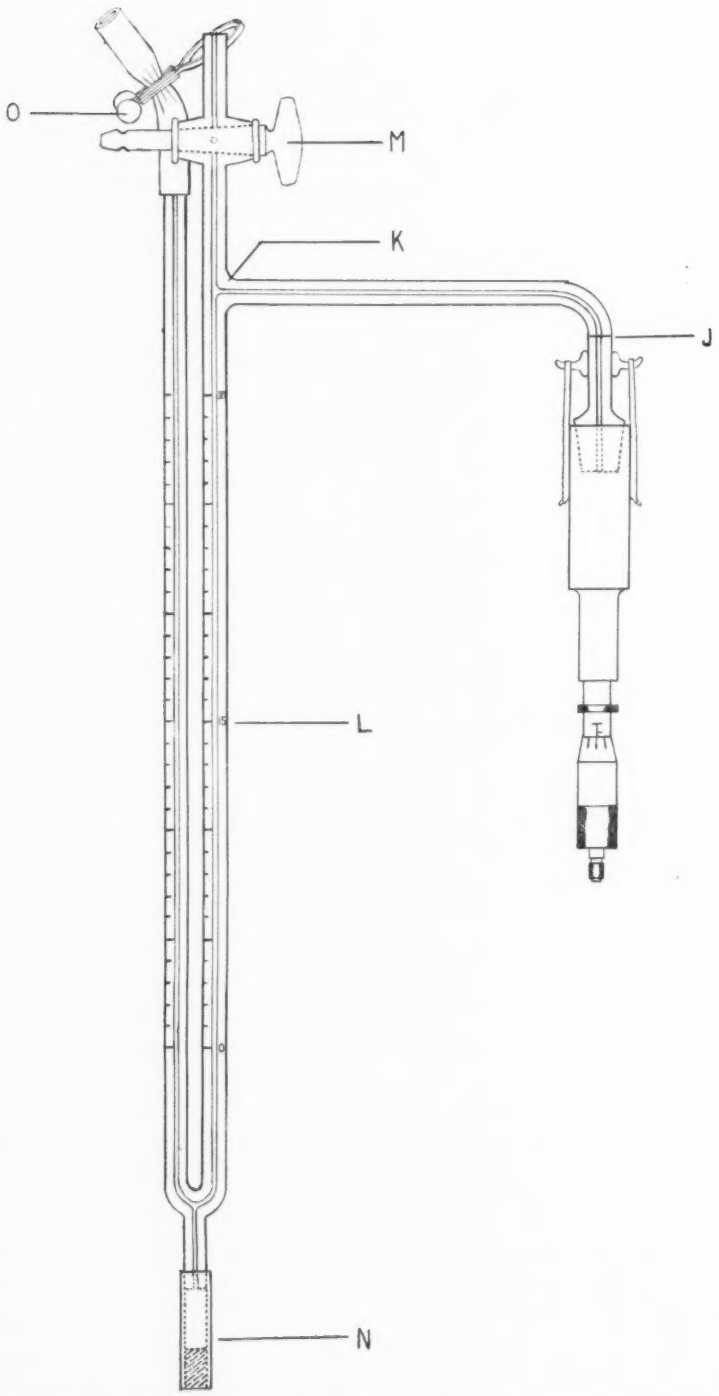


Fig. 2.—Calibrator inserted on Warburg manometer (for explanation see text).

and the plunger is advanced until the water meniscus reaches point *K* (Fig. 2). Further advancement of the water meniscus fills the capillary between point *K* and the stopcock (*M*). When water reaches the bore of the stopcock (*M*), the stopcock is closed and the pinch clamp (*O*) is opened. The liquid is advanced to the 15 cm. mark (*L*) of the manometer. The micrometer is again read and the volume determined by subtracting the initial from the final reading. The liquid is then removed from the manometer by reversing the foregoing procedure and the calibration may be repeated.

Results of several calibrations are shown in Table I. It will be noted that the volume of the manometer as determined in the first trial is slightly greater than that observed in subsequent trials. This difference is due to the residual water which is left in the manometer after the first calibration. It is further

TABLE I. WARBURG MANOMETER CALIBRATION

| REMARKS | TRIAL | VOLUME (C.C.) | | |
|---|---|---|---|---|
| | | 23 | 28 | 34 |
| Manometer dry | 1 | .746 | .803 | .839 |
| Manometer wet | 2 | .720 | .787 | .820 |
| Manometer wet | 3 | .723 | .789 | |
| Water left in manometers | | .025 | .014 | .019 |

noted that repeated runs on a wet manometer differ by less than .005 cubic centimeter. The accuracy of the volume measurement obtainable with this calibrator far exceeds the accuracy required for the calculation of the manometer constant. The accuracy of the calibrator itself may be checked by inserting a 2 c.c. pipette through a one-holed rubber stopper and inserting the stopper into the standard taper (*H*) of the calibrator. Since the plunger has a cross-sectional area of exactly 1 sq. cm., each millimeter advancement of the micrometer plunger corresponds to 0.100 cubic centimeter. Since it takes two revolutions of the micrometer to advance the spindle 1 mm. and since there are fifty small divisions per revolution of the micrometer, each small division of the scale of the micrometer corresponds to a volume of 0.001 cubic centimeter.

## SUMMARY

A simple, rapid calibrator for the Warburg manometer is described.

# A NEWLY DEVELOPED ELECTROMAGNETIC FLOW METER

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THE general principle of an electromagnetic flow meter is not new. It was first developed by Kolin[1] in this country and independently by Wetterer[2] in Germany. Kolin[3, 4] developed both an A.C. model and a D.C. model; the latter was subsequently modified by Jochim.[5] These methods featured sleeves of various design which contained electrodes in positions so that when the sleeve surrounded a blood vessel, the electrodes were in contact with the outside surface of the blood vessel. While such devices possessed the advantage of not requiring incision into the blood vessel, they incurred the disadvantages of voltage drift and minor artifacts due to vessel drying, varying vessel diameter, and extraneous potential pickup. In order to avoid these disadvantages, a new method of potential pickup was developed in this laboratory which has proved exceedingly practical for the measurement of blood flow when used with an A.C. amplifier and suitable recorder. This newly developed electromagnetic blood flow meter has been found to be unusually accurate in its measurement of blood flow and to possess negligible voltage drift.

## PRINCIPLE

The basic principle upon which the operation of an electromagnetic flow meter depends is that when an electric conductor moves across the lines of force of a magnetic field, a potential difference is created in the conductor. If the field is uniform, the conductor moves in a plane at right angles to the magnetic field and the length of the conductor extends at right angles to both the field and the direction of motion; the resulting electromotive force will be directly proportional to field strength, speed of the conductor, and length of the conductor within the field. Therefore:

$$E = B \cdot l \cdot v \cdot 10^{-8}$$

where E is the potential difference in volts; B, field strength in gauss; l, length of the conductor within the field in centimeters (across the electrodes); and v, speed of the conductor in centimeters per second. By use of the A.C. system, including an A.C. magnet, there is an additional constant to be accounted for because of the electrodes and their leads acting as a one-turn transformer. Therefore, the total equation of an A.C. system must be altered to read as follows:

$$E = B \cdot l \cdot v \cdot 10^{-8} + A$$

where "A" voltage is the potential developed by the electrodes and leads, this voltage being proportional to B field strength. In order to cancel out a portion of "A" voltage, on the magnet a turn is made which is phased oppositely to that of the leads. The canceling potentiometer is turned in a manner to diminish "A" voltage to a minimum. Once

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Received for publication, Aug. 10, 1949.

this point is established, the voltage is of a constant and easily accountable magnitude. If B and l are kept constant, K may be stated:

$$K = B \cdot l \cdot 10^{-8}$$

and

$$E = K \cdot v + A$$

As shown by the equations, when the voltage "A" is subtracted, the electromotive force is a function of the single variable, the velocity of flow, which theoretically is linear. However, for practical considerations one is interested in volume flow in cubic centimeters per minute, rather than velocity flow in centimeters per second. These quantities are related as follows:

$$Q = 60 \cdot v \cdot \frac{2 \cdot \pi \cdot d^2}{4}$$

where Q is the volume flow in cubic centimeters per minute; v, the velocity flow in centimeters per second; and d, the diameter of the tube or vessel. While this formula shows the relations of quantities, it is not required for the use of the newly developed meter because, due to the manner of its construction, calibrations may be made of actual flow during an increment of time to read directly as cubic centimeters per minute blood flow.

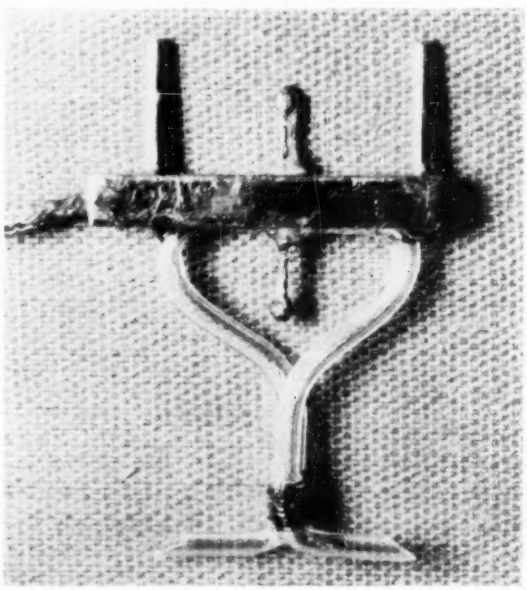

Fig 1.—Cannula used with electromagnetic blood flow meter.

## MATERIALS AND METHODS

*Description of Apparatus.*—The complete flow meter consists of a specially constructed cannula, an A.C. magnet, an A.C. amplifier, and an Esterline-Angus 5 Ma. recorder.

The construction of the cannula consists of a glass or plastic tube of previously determined diameter to equal the size of the lumen of the blood vessel to be cannulated. The cannula and pickup shown in Fig. 1 has a diameter of 3 millimeters. This particular cannula which is designed to fit the femoral artery of a dog is 4 cm. in height and 2.5 cm. in width; the bottom forms an anchorage to secure ligatures. Fig. 2 better reveals the detailed construction. In the top of the cannula are inserted two tungsten (platinum may be used) wire rods with rounded tips so that the tips just touch the periphery of the

lumen. Onto these tips just outside of the glass are soldered the two pickup leads which are then tightly twisted and passed through the center of the housing and into a shielded cable which runs to the amplifier connection. On the top of the housing are two pins which fit into the magnet case in order to assure identical positioning of the cannula during each measurement.

The housing is filled with Lucite for the purpose of stabilizing the position of the lead wires in the magnetic field. This is done by dissolving a portion of Lucite in $CHCl_3$, pouring the solution in the housing around the cannula and leads, and letting it congeal to form solid Lucite. This operation also makes the housing repellant to liquids. The

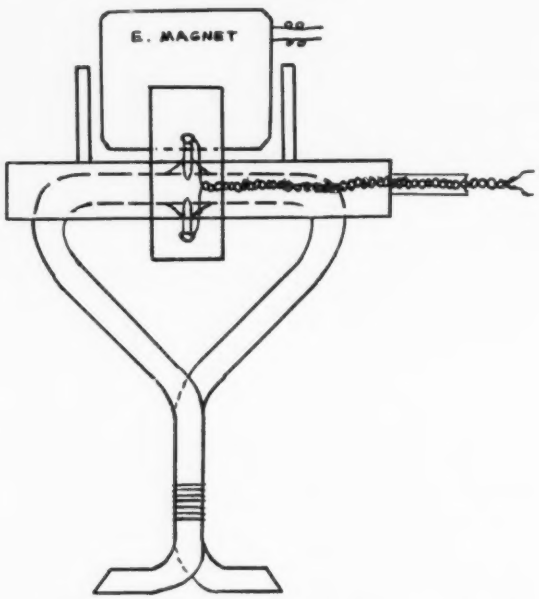


Fig. 2.—Diagram of cannula and pickup leads showing the position of the magnet.

slender base of the cannula is wrapped tightly with a single layer of No. 8 linen thread and coated with the liquid plastic to add strength to the cannula. Care is taken in the construction of the cannula that there are no constrictions in the lumen and that there are no sharp bends. The short section across the top which holds the electrodes is straight.

The cannula and pickup incorporates the following features: (a) an electrical system well isolated from the surrounding tissues; (b) a lumen of uniform size to match the size of the lumen of the blood vessel measured, with no constrictions in its length; (c) a minimum of glass or plastic surface (less than four inches in length) with which the blood is in contact; (d) a reinforced slender base around which to secure ligatures surrounding the ends of the arteries; and (e) a specially designed bronze housing surrounding the electrodes through which the tightly twisted pickup leads are inserted to traverse the center of the magnetic field and be shielded from contact with the magnetic poles. It is the design of this housing which allows a minimal and invariable background pickup and thus prevents voltage drift on the recorder.

The A.C. magnet used with the meter is made from stacked laminations of ½ inch 29 gauge L-4 audio A iron. The coil is made of 1,800 turns of No. 25 wire excited with a 110 V. A.C. 60 c.p.s. source. This gives a field of about 1,000 gauss across a ½ cubic inch air gap between pole tips. A 60 c.p.s. power source limits the frequency response of the whole system to fluctuations of less than 60 c.p.s., but it is possible to modify this limitation by changing the carrier frequency to 400 c.p.s. if desired. Should the meter be used

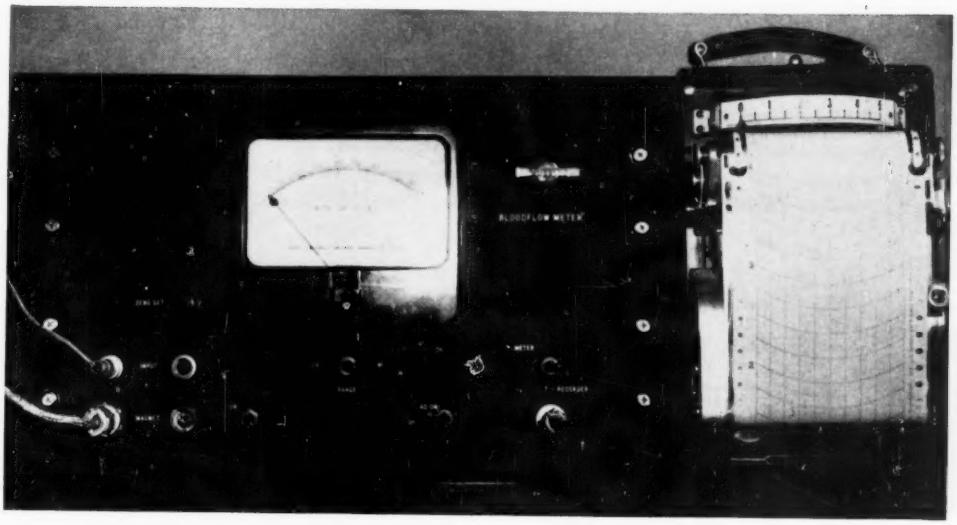

Fig. 3.—Recorder and amplifier showing the connections as labeled on the panel.

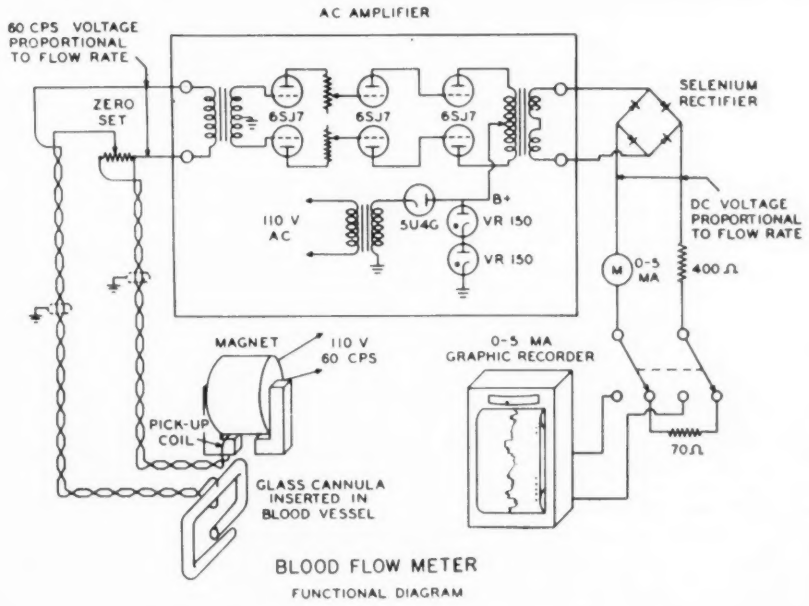


Fig. 4.—Simplified diagram showing the electromagnetic pickup, amplifier, and recorder.

with a high frequency response recorder, this modification is recommended. For convenience in affixing the position of the magnet, a screw-type lift with a crank attached is used to slowly lower the magnet into position over the cannula. The magnet is held by a ball-in-socket swivel which will allow it to be adjusted at a convenient angle.

The A.C. amplifier and recorder used are shown in Fig. 3. The amplifier is adapted from the original A.C. amplifier of Kolin, with minor modifications. Three stages of 6SJ7 tubes are used for the principal circuit with two VR 150 tubes being used on the B+ circuit.

This amplifier has proved remarkably stable and features two input channels for pickup, each connected to a potentiometer to be utilized in canceling out stray background. A selenium rectifier is used in the output circuit. Any stable amplifier with sufficiently high gain would be adaptable for the electromagnetic flow meter, so long as it matches the characteristics of the pickup and the magnet used. This particular amplifier is most adaptable to a 60 c.p.s. magnet. The amplifier used here has two gain settings, the two amplifications giving a total range of blood flow measurement from zero to 300 c.c. per minute with different cannulae. The range of measurement may be increased by constructing the

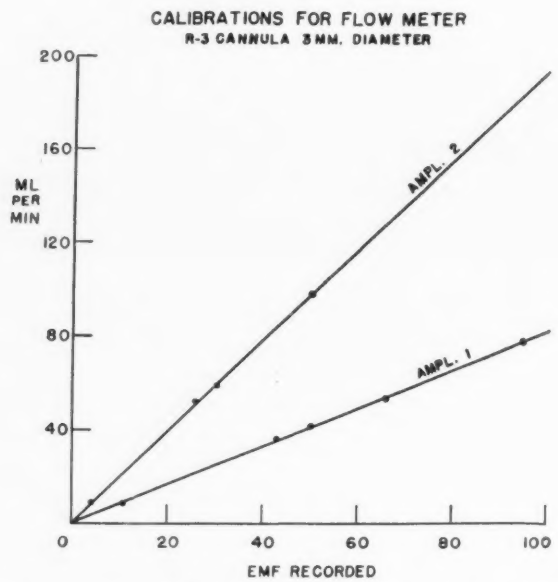


Fig. 5.—Sample calibration curve showing the use of two amplifications with one cannula.

electrodes of the cannula so that the distance between the tips is diminished. Conversely, greater sensitivity is achieved by constructing the electrodes of the cannula so that the distance between the tips is greater. In all cases the lumen of the cannula should be as nearly as possible identical with the size of the blood vessels.

A 5 Ma. Esterline-Angus recorder is used on the electromagnetic flow meter as a compromise choice of various possibilities. This instrument requires 5 Ma. through an internal resistance of 70 ohms for full scale deflection. This is a heavily damped instrument and will not record events accurately under 200 milliseconds but this feature is of value in the recording of mean blood flow where a concomitant record only of heart rate and respiration is desired. When events of shorter duration are of interest, a recorder of higher frequency response should be employed. Fig. 4 reveals a functional diagram of the entire apparatus as it is operated showing the position of the magnet, the general structure of the amplifier, and the recorder connections.

*Calibration of the Instrument.*—Although a force-pump perfusion system has been used to calibrate the flow meter, it is felt that the preferred method is to measure the flow of blood out of a blood vessel directly into a graduate over a convenient period of time. This method is to be desired for its simplicity and the elimination of extraneous intervening variables. In operation, the distal end of the cannula is attached to a plastic tubing of the same size which runs to a graduate. A previously applied clamp on the proximal artery is released for one minute so that a one-minute flow of blood is spilled into the graduate. A point is made on the midline of the excursions on the record which corresponds to the milliliters of blood

measured. This is one point on the calibration curve. By constricting the end of the plastic tubing, lower rates of flow may be measured until a number of desired points on the flow curve may be calibrated. A curve of best fit may be drawn transecting the points and may be extrapolated to the base line. This point on the base line, or "true base" will not fall on zero, but upon an electromotive force reading which represents the "A" voltage previously described. These readings may be made identical, by means of the potentiometer adjustment. Fig. 5 shows typical calibration curves by use of two amplifications. In this calibration graph, the "A" voltage has been subtracted and it may be seen that the curves are, for all practical purposes, linear.

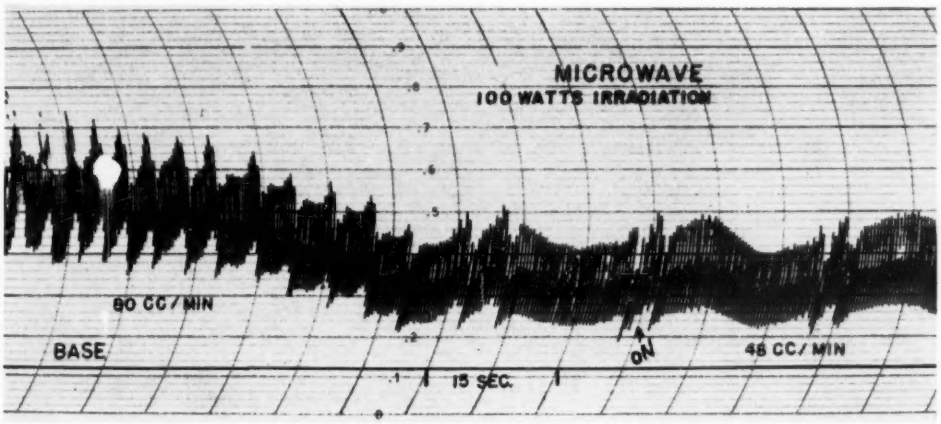


Fig. 6.—Sample record made with the new electromagnetic flow meter. The record reads from right to left.

Once it is calibrated, a cannula does not require recalibration unless it develops a fracture or similar defect. However, because of such possibilities, frequent recalibration may be advisable. The typical observation with a fractured cannula is an enormous base voltage which ordinarily can be detected readily.

## RESULTS

Fig. 6 shows a typical blood flow record which reads from right to left. This sample record shows the results of 12 cm. microwave irradiations using 100 watts output upon the limb of a dog. The wave director was placed close to the surface of the limb. In the figure, the heavily inscribed base line represents the background pickup, or "A" voltage, so that voltage induced by blood flow reads from this point to the midline of the excursions to represent mean flow. This point at the midline was arbitrarily selected when calibrating the cannula. When this distance as read on the ordinate is multiplied by the k-value of the cannula, in this case $(2.0 \times 10^2)$, a figure is derived which represents blood flow in cubic centimeters per minute. For example, the control flow may be calculated as follows:

$$0.24 \times 2.0 \times 10^2 = 48 \text{ c.c./min.}$$

The calibration should be experimentally established as suggested, and the "A" voltage reading may be conveniently adjusted. Since the "A" voltage will be 90° out of phase with the flow voltage, they cannot be added algebraically.

Fig. 6 is a reproduction of blood flow in the femoral artery. The rapid oscillations are the result of the cardiac rhythm, while the slower oscillations are due to respirations. These events are reflected in the flow record with sufficient clarity that the record may be used to count the respirations and heart rate. It may be observed that while the rate of blood flow and respiration have been increased by the irradiations, there is not noticeable increase in heart rate. This record was made at one of the intermediate speeds. A faster speed makes the excursions much easier to analyze, while a slower speed achieves a greater economy of record.

## DISCUSSION

While it is essentially a pulsatile flow meter, this device as described here is best adapted for the measurement of mean blood flow. As previously suggested, it is possible to better adapt this meter for the measurement of pulsatile blood flow where a finer analysis of events of a short duration is desired.

The meter as described has proved exceedingly capable for the measurement of mean blood flow in both veins and arteries. It has been found to possess less than 5 per cent error in measurement under physiologic conditions and has been operated for four- to six-hour periods with negligible voltage drift. A criticism of this type of flow meter is that general anesthesia and an anticoagulant must be used because the blood vessel is cannulated. On the other hand it is simple to operate, is substantially free from subjective factors, and possesses a relatively linear calibration curve. By use of different cannulae, the range of flow measurement is from 0 to 300 c.c. per minute. This range may be expanded with simple modifications. The meter may be easily cleaned following each use by means of a detergent and distilled water. This cleaning, carefully done, is important for proper measurements.[6] In brief, while the development of this meter entailed certain compromises it is felt that it has proved to be a practical, accurate, and easily operated device for the measurement of blood flow under many conditions encountered in laboratory measurement.

## SUMMARY

A new electromagnetic blood flow meter has been developed which produces a continuous permanent record of mean blood flow. This device is adaptable to measure pulsatile blood flow in the respect that it readily records the effect of the heart rhythm and respiration upon blood flow. Modifications are suggested for recording events of shorter duration. The use of this meter entails the disadvantages inherent with cannulation, but such disadvantages must be weighed against the advantages of accuracy, stability, and ease of operation. The stability and accuracy of this meter is in a large measure dependent upon careful construction of the cannula and pickup leads.

The authors gratefully acknowledge the cooperation and assistance of Dr. J. W. Clark in the development of this work.

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# EVALUATION OF A NEW CAPILLARY RESISTOMETER: THE PETECHIOMETER

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## INTRODUCTION

CAPILLARY resistance determinations were made on one hundred patients with two different types of suction apparatus and the readings compared. A modified Dalldorf resistometer was used on one forearm as previously described,[1] and a new resistometer, the Petechiometer,* was applied to the other forearm.

## MATERIALS AND METHODS

Capillary resistance is the minimal amount of suction applied to the skin for one minute capable of producing one petechia or more. It is measured in centimeters of mercury negative pressure or suction. With the modified Dalldorf resistometer, readings were made at intervals of 5 cm., the amount of suction being read directly from a vacuum gauge. The Petechiometer, on the other hand, has no vacuum gauge but is adjusted to deliver 10, 20, and 30 cm. of mercury suction. The bell of the old resistometer is 1 cm. in diameter while that of the Petechiometer is 2 centimeters.

In all of the one hundred patients two readings were noted, the old instrument being used on one forearm and the Petechiometer on the other, Fig. 1. Because in some studies[2, 3] readings were normally slightly lower on the right arm, it was decided to reverse machines in the second group of fifty patients. The 200 capillary resistance readings may be compared in Table I.

*Estimation of Capillary Resistance With the Petechiometer.*—The plunger is grooved at three points, each yielding a different suction. The adjustable stop ring is inserted in the innermost groove. One then places water just below the antecubital space before applying the bell to the wet skin. The air is expelled by pressure of the right thumb on the end of the plunger and the bell placed on the skin. The bell is held in contact with the skin lightly, but firmly, with pressure from the index finger of the left hand upon the top of the bell. As the thumb pressure on the plunger is released quickly a spring action applies suction to the skin area. Suction is applied for one minute and then released by pushing inward on the plunger. After removing the Petechiometer, thirty seconds are allowed before looking for petechiae. If no petechiae are found, the test is repeated using each of the stop-ring settings, i.e., the innermost yielding 10 cm. mercury suction, the middle setting 20 cm. mercury suction, and the outermost slot setting 30 cm. mercury suction. The determinations are not made closer together than the diameter of the suction bell.

Received for publication, Aug. 19, 1949.

*The Petechiometer is the registered trade mark owned by the Rexall Drug Company. The Petechiometer consists of a small suction pump with a plunger and a clear plastic Bier bell, or suction cup, 2 cm. in internal diameter, with a magnifying glass blown into the upper surface of the cup to facilitate in situ reading of the petechiae developed.

The number of petechiae produced by the Petechiometer was noted. In the final comparison of results, this procedure proved valuable, for it permitted interpolation to intervals of 5 cm. when excessive numbers of petechiae were produced at the 10, 20, or 30 cm. readings. For example, Patient 29 showed a capillary resistance of 10 cm. on the Petechiometer, but had forty petechiae.

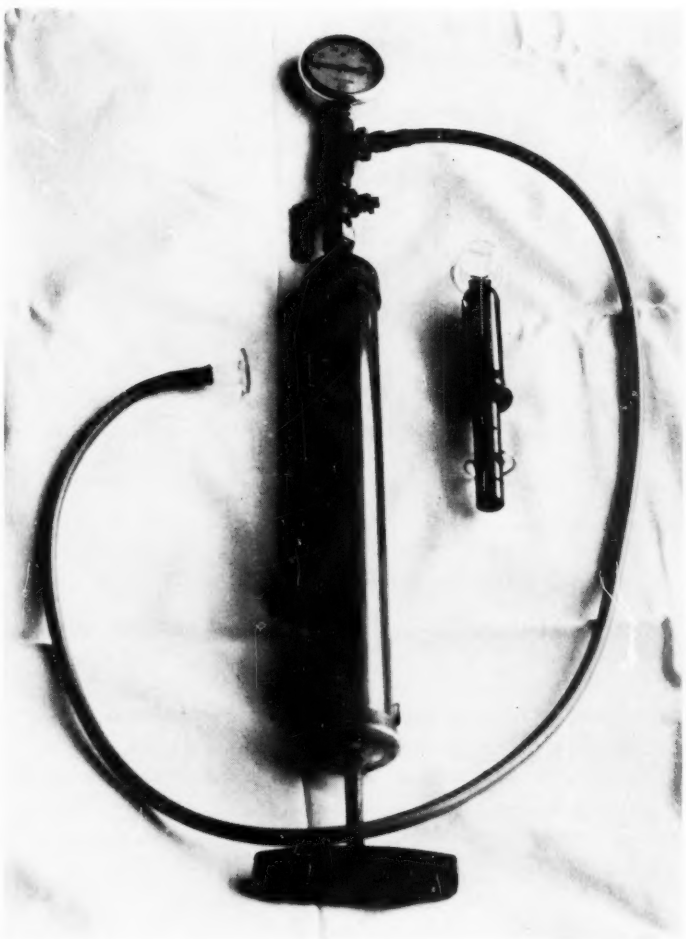

Fig. 1.—Comparison between old and new resistometers.

Obviously, because of the many petechiae produced, the true capillary resistance was actually less than 10 centimeters. Using the old resistometer, the capillary resistance in this patient was 5 centimeters. When comparisons were made with the two machines, it was found that when six or more petechiae were produced by the Petechiometer, one could in most instances subtract 5 cm. from the capillary resistance shown by this new instrument and approximate the reading given by the old machine.

TABLE I. CAPILLARY RESISTANCE READINGS (CENTIMETERS OF MERCURY SUCTION) IN 100 PATIENTS WITH NEW AND OLD RESISTOMETERS, USING RIGHT AND LEFT FOREARMS

| | NEW RIGHT | | | OLD LEFT | | NEW RIGHT | | | OLD LEFT | | NEW LEFT | | | OLD RIGHT | | NEW LEFT | | | OLD RIGHT |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | * | P | † | | | * | P | † | | | * | P | † | | | * | P | † | |
| 1. | | | 20 | 15 | 26. | | | 10 | 10 | 51. | | | 30 | 15 | 76. | | | 30 | 30 |
| 2. | 10 | 8 | 5 | 5 | 27. | | | 20 | 10 | 52. | | | 10 | 10 | 77. | 20 | 8 | 15 | 15 |
| 3. | | | 10 | 5 | 28. | 10 | 12 | 5 | 5 | 53. | | | 20 | 20 | 78. | 10 | 8 | 5 | 5 |
| 4. | | | 20 | 15 | 29. | 10 | 40 | 5 | 5 | 54. | 10 | 20 | 5 | 10 | 79. | 10 | 7 | 5 | 5 |
| 5. | | | 10 | 10 | 30. | | | 20 | 25 | 55. | | | 10 | 10 | 80. | 10 | 8 | 5 | 15 |
| 6. | | | 20 | 15 | 31. | | | 10 | 10 | 56. | | | 10 | 15 | 81. | | | 10 | 10 |
| 7. | | | 20 | 15 | 32. | | | 30 | 15 | 57. | | | 10 | 15 | 82. | | | 10 | 15 |
| 8. | 20 | 8 | 15 | 20 | 33. | | | 10 | 10 | 58. | | | 20 | 20 | 83. | 10 | 7 | 5 | 10 |
| 9. | | | 20 | 15 | 34. | | | 20 | 25 | 59. | | | 10 | 15 | 84. | | | 20 | 15 |
| 10. | | | 20 | 25 | 35. | | | 20 | 15 | 60. | | | 10 | 5 | 85. | | | 10 | 10 |
| 11. | | | 10 | 15 | 36. | | | 20 | 15 | 61. | | | 10 | 5 | 86. | | | 10 | 10 |
| 12. | | | 20 | 20 | 37. | | | 20 | 15 | 62. | | | 10 | 15 | 87. | | | 20 | 10 |
| 13. | | | 10 | 10 | 38. | 10 | 7 | 5 | 10 | 63. | | | 20 | 15 | 88. | 10 | 6 | 5 | 5 |
| 14. | | | 20 | 25 | 39. | 20 | 6 | 15 | 20 | 64. | | | 20 | 15 | 89. | 10 | 8 | 5 | 10 |
| 15. | | | 20 | 20 | 40. | | | 10 | 15 | 65. | | | 10 | 10 | 90. | | | 10 | 10 |
| 16. | | | 20 | 15 | 41. | 30 | 14 | 25 | 25 | 66. | | | 10 | 10 | 91. | | | 15 | 20 |
| 17. | | | 20 | 15 | 42. | | | 20 | 25 | 67. | 20 | 8 | 15 | 15 | 92. | | | 10 | 10 |
| 18. | 20 | 8 | 15 | 30 | 43. | 20 | 8 | 15 | 15 | 68. | 10 | 6 | 5 | 5 | 93. | | | 10 | 10 |
| 19. | | | 30 | 25 | 44. | | | 10 | 10 | 69. | | | 20 | 25 | 94. | | | 10 | 10 |
| 20. | 20 | 12 | 15 | 25 | 45. | 20 | 8 | 15 | 15 | 70. | 20 | 8 | 15 | 15 | 95. | | | 10 | 15 |
| 21. | | | 10 | 10 | 46. | | | 10 | 20 | 71. | 20 | 8 | 15 | 15 | 96. | | | 30 | 20 |
| 22. | | | 30 | 35 | 47. | | | 20 | 30 | 72. | | | 20 | 25 | 97. | | | 10 | 15 |
| 23. | | | 20 | 25 | 48. | | | 10 | 10 | 73. | 20 | 8 | 15 | 15 | 98. | 20 | 16 | 15 | 2 |
| 24. | | | 10 | 15 | 49. | 10 | 6 | 5 | 5 | 74. | | | 20 | 20 | 99 | 10 | 17 | 5 | 5 |
| 25. | | | 10 | 10 | 50. | | | 10 | 5 | 75. | 20 | 15 | 15 | 15 | 100. | | | 20 | 15 |

*Capillary resistance before 5 cm. correction for excessive number of petechiae (six or more).
P, Number of petechiae.
†Corrected capillary resistance.

## RESULTS

By subtracting 5 cm. when six or more petechiae were noted at the capillary resistance determination with the Petechiometer, the average reading with the old instrument was 14.75 cm. and with the Petechiometer 14.25 centimeters. This similarity is not so constant in individual readings. Only 43 per cent showed no difference between old and new resistometers and 90 per cent did not vary more than 5 centimeters. Of the ten patients who differed by more than 5 cm. between arms, seven patients showed a 10 cm. difference and three patients a 15 cm. difference. One may expect, however, to find similar variations between arms even when using the same resistometer.[2, 3]

## DISCUSSION

A simple capillary resistometer to determine bleeding tendencies should be welcomed by physicians. It has been noted that the lower the capillary resistance, the greater is the bleeding time.[4] A capillary resistance of 5 cm. means marked fragility. Capillary fragility accounts for bleeding in such conditions as purpura and rheumatic fever and in hypertensive patients.[3, 5, 6]

Without a suction apparatus for determining capillary resistance, the clinician may resort to positive pressure methods, as by use of the sphygmomanometer.[7, 8] The disadvantages of such methods are listed by Tey[9] as follows: (1) determination is time consuming; (2) test cannot be repeated for days;

(3) there is no gradation in the same subject; (4) results cannot be read with accuracy; (5) positive pressure methods are unsuited for pharmacologic investigation, where several determinations must be made within a few minutes since only two body surfaces (arms) are available.

On the other hand, use of the Petechiometer is simple and painless. Determination of capillary resistance requires less than five minutes. The instrument is small and can be carried in one's bag.

## CONCLUSIONS

Capillary resistance determinations were made on one hundred patients, using a modified Dalldorf resistometer with vacuum gauge on one forearm and a new instrument, the Petechiometer, on the other.

Although readings on both forearms differed in individual subjects, these differences were usually slight. Only 10 per cent of patients showed readings which differed more than 5 cm. between arms. Minor differences are normally noted between arms. These differences tended to balance in the final compilation, for the average capillary resistance with the old machine was 14.75 cm. and with the Petechiometer 14.25 centimeters. The Petechiometer thus appears to be accurate.

The Petechiometer has the following advantages over the older resistometer: it is on the market; it is simple to use; it is compact and small.

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# A SIMPLIFIED VENOUS OCCLUSION METHOD OF DIGIT BLOOD FLOW ESTIMATION USING THE BURCH-WINSOR PLETHYSMOGRAPH

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DIGIT blood flow studies based on the venous occlusion principle have been greatly simplified by the development of a clinical type of transmission plethysmograph by Burch.[1] At the present time venous occlusion blood flow determinations are being carried out as a part of the regular digit plethysmographic study on both fingers and toes in our laboratory. The basic details of this type of study have been recorded by Wilkins,[2] Goetz,[3] Abramson,[4] and others.[6] Our purpose in reporting this method is to emphasize the ease with which venous congestion methods may be adapted to the Burch-Winsor plethysmograph. Measurements with this type of apparatus depend upon volume changes in the part under study, the variables being amount and rate of volume change. For the most part an increase in the volume of a trapped part is dependent upon either increased arterial inflow or decreased venous outflow or both. If one occludes the venous outflow without disturbing the arterial inflow the volume change resulting represents an indirect estimate of the arterial inflow. The inherent limitations of this type of determination have been adequately discussed by Abramson and by others.[2, 3, 4, 6] We are aware of these limitations, but with increasing experience we are coming to feel that digit flow determinations are worth-while adjuncts to digit plethysmography, particularly in attempting to decide about the presence or absence of vasomotor activity in the digital blood supply.

The problem of applying an occluding pressure to the base of the digit might be mentioned first. Our first attempt was a brass-backed latex ring. This was a satisfactory method for fingers but in the case of toes a ring large enough to pass over the end of the toe was frequently too large to satisfactorily compress the toe at its base. The brass-backed latex cuffs were discarded in favor of Abramson's adhesive tape-latex tube method. In making the latex tubing for the occluding cuffs a glass rod 6 mm. in diameter which has been dusted with talcum powder is dipped into a container of prevulcanized liquid latex two or three times. After several hours of drying, the surface of the rubber on the glass rod is dusted with talcum powder and the rubber is rolled off the glass. The tubing thus obtained is thin enough to avoid bulk and strong enough to withstand the occluding pressures. The latex tubing is attached to a small catheter or plasma tubing with a fine silk binding re-enforced with a turn of adhesive tape. The latex tubing is then backed with ordinary adhesive tape.

From the Department of Surgery, Boston University School of Medicine and The Massachusetts Memorial Hospitals.
Received for publication, Aug. 20, 1943.

The end is turned under and covered with a tab of adhesive. The completed cuff is 8 to 10 cm. in length and approximately 1 cm. wide.* These cuffs, if well made and free of bubbles, will survive a week or more of active use. In applying the cuff to the digit, the cuff is wrapped around the base of the digit and a short piece of adhesive is used to secure the cuff to itself to prevent slipping. (Fig. 1, *A* and *B*.)

The method of cuff inflation used is a simple pressure bottle arrangement similar to that of Goetz.[3] The essentials of the pressure system are a 1 or 2 liter widemouthed bottle, a tightly fitting rubber stopper, a large three-way glass stopcock, some type of manometer—either anaeroid or mercury, a blood pressure type of compression bulb, and suitable tubing and glass or metal connections. The illustrations (Fig. 2, *A* and *B*) are self-explanatory and give a satisfactory scheme of connections for use.

In use the occluding pressures should be well below diastolic arterial pressure and we have found 60 to 70 mm. (Hg) to be satisfactory for most digits. When air is allowed to flow into a single cuff, the others having been excluded by clamps, the bottle pressure falls 1 to 2 mm. of mercury with each inflation. This is corrected prior to each occlusion by means of the compression bulb.

To summarize the steps in the digit flow determination:

1. The digit part is measured in the displacement tube apparatus[1] to a volume of 5 cubic centimeters. This volume is indicated on the digit by a weak gentian stain which is employed as the "measuring fluid."

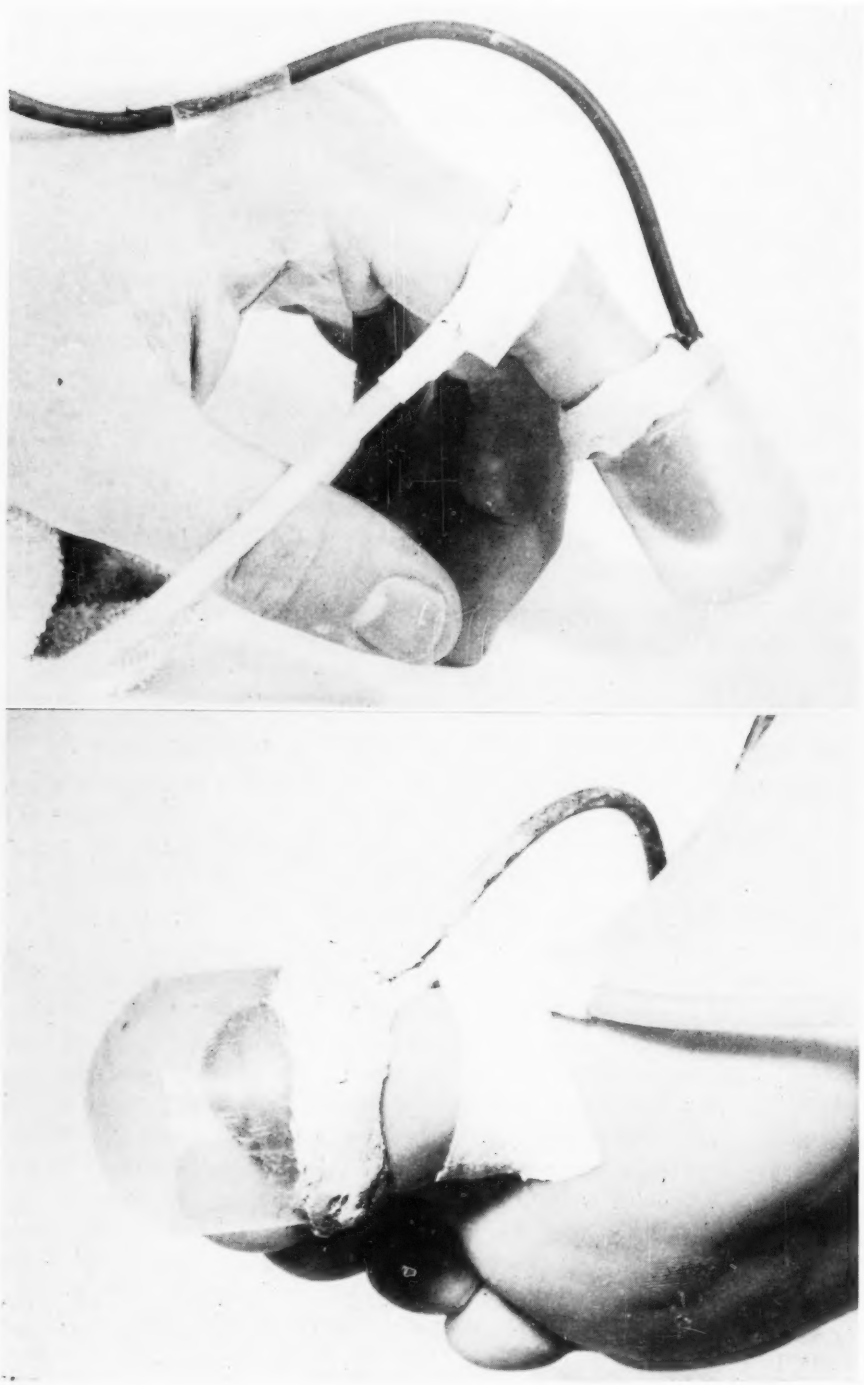
2. The extremity plastic cup is sealed in place at the proximal edge of the gentian stain with the sealing compound Kalk-Kord, previously reported.[5]

3. After the cup seal has been tested, the occluding cuffs are applied to the base of the digit in such a way that there is no contact with the cup or the sealing compound—and no contact between the tubing from the cup and cuff. Trial cuff inflations are then carried out to make certain the cuffs are in the best alignment to prevent unnecessary motion of the digit when the cuff pressure is introduced.

4. String sensitivity is adjusted to move 10 mm. per 10 mm.[3] volume charge with the calibration lever. The routine plethysmographic tracings are made at slow speed, standardizing each run twice. Following the second calibration, the base line is shifted to the left side of the aperture and the camera speed shifted to "fast" just prior to the application of cuff pressure. This will require a certain amount of practice by operator and assistant. When the maximal base line shift (i.e. volume change) has occurred, the camera is shifted to slow speed and cuff pressure is released by turning the stopcock to the "exhaust" position. The base line is adjusted to the left side of the aperture, if necessary, following which the venous occlusion test is repeated. The camera is then stopped and the next digit is prepared for recording. With practice, the volume pulses and two venous occlusion runs can be recorded on about 12 inches of paper for each digit.

*The latex which we have found satisfactory for this use is a formula designated No. GL-15c, furnished by the General Latex and Chemical Corp., 666 Main Street, Cambridge, Mass.

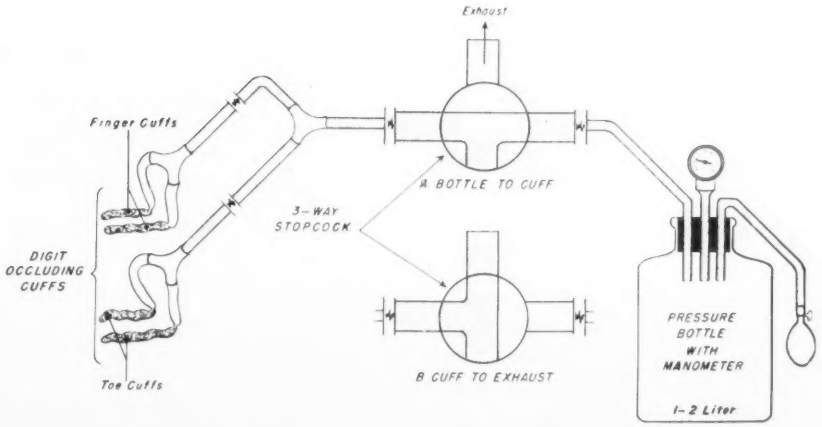
*A.*



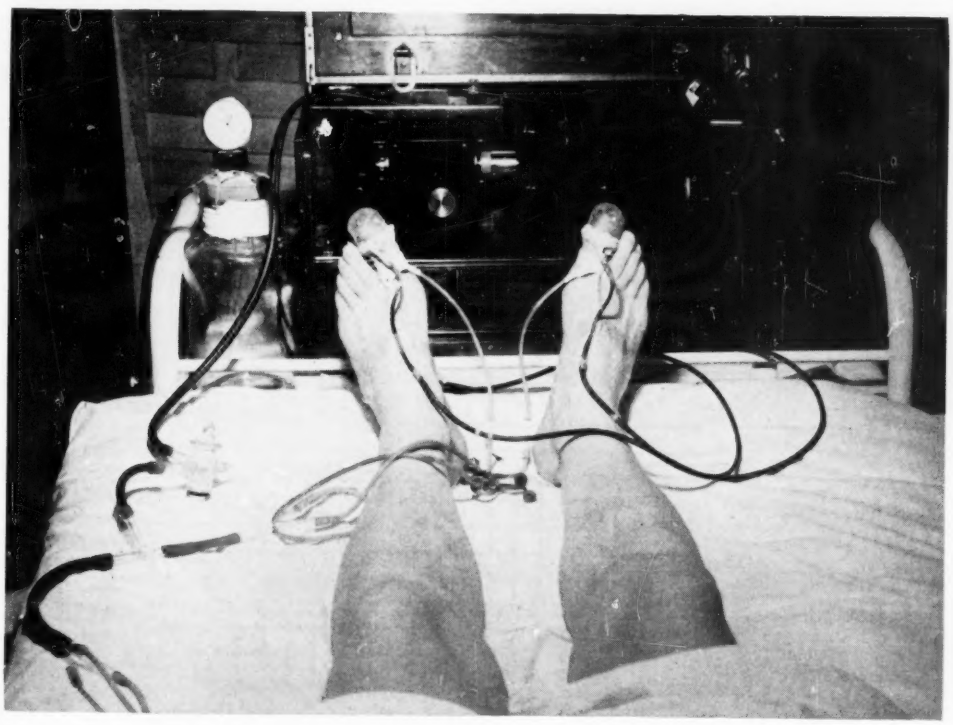
*B.*

Fig. 1.—See text for explanation.

DIAGRAMMATIC SYSTEM
OF CUFF-PRESSURE BOTTLE CONNECTIONS

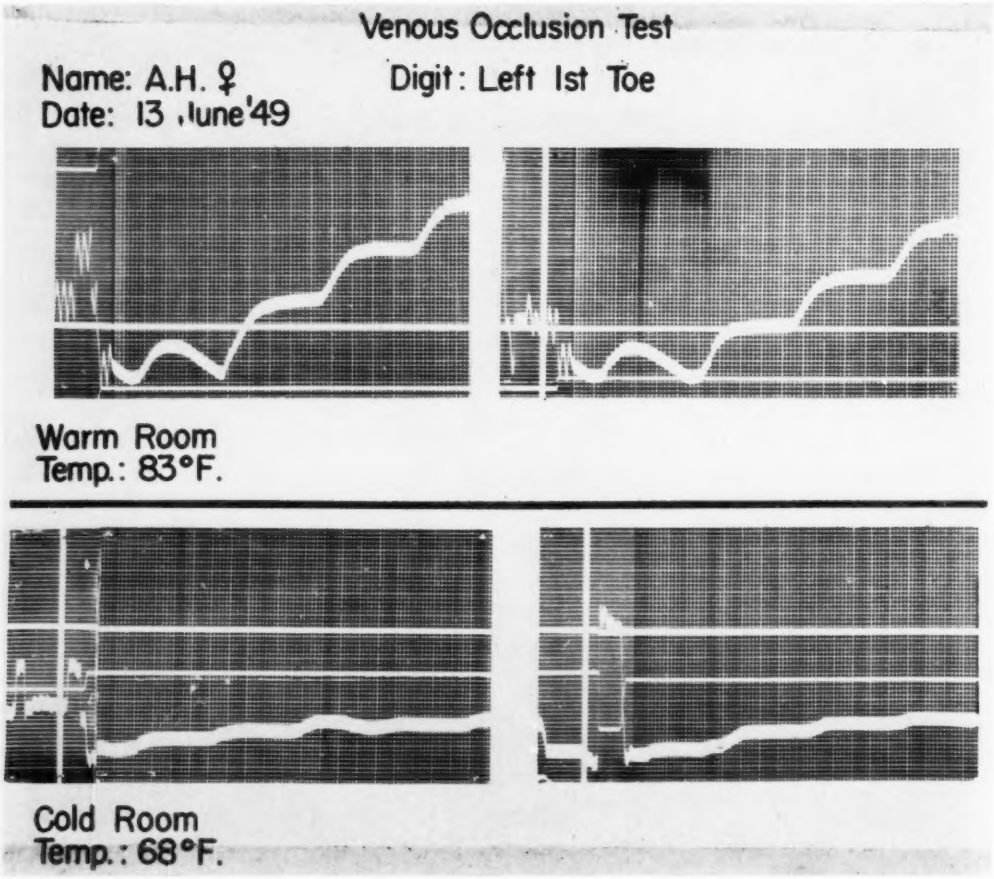



*A.*

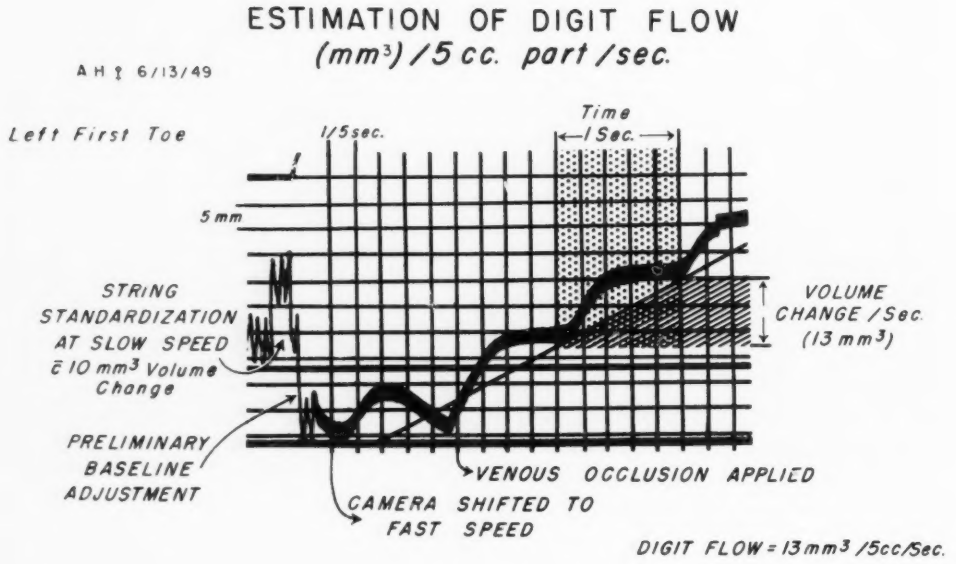


*B.*

Fig. 2.


Venous Occlusion Test
Name: A.H. ♀
Date: 13 June '49
Digit: Left 1st Toe
Warm Room
Temp.: 83°F.
Cold Room
Temp.: 68°F.

*A.*


ESTIMATION OF DIGIT FLOW
(mm³) / 5 cc. part / sec.
A H ♀ 6/13/49
Left First Toe
1/5 sec.
Time
1 Sec.
5 mm
STRING STANDARDIZATION AT SLOW SPEED ≅ 10 mm³ Volume Change
VOLUME CHANGE / Sec. (13 mm³)
PRELIMINARY BASELINE ADJUSTMENT
VENOUS OCCLUSION APPLIED
CAMERA SHIFTED TO FAST SPEED
DIGIT FLOW = 13 mm³ / 5cc / Sec.

*B.*

Fig. 3.

5. After the tracing has been developed, the digit flow can be readily determined by measuring the amount of volume change per unit of time (Fig. 3, *B*). We have arbitrarily used millimeters[3] per second per 5 c.c. of part. In drawing the tangent slope line it is desirable to avoid using the first pulse excursion if possible since positive or negative artifacts are common. In rapid flows this may not be possible because of the speed with which the string shadow crosses the entire width of the paper, in which case the slope line should be placed along the most representative portion of the curve. In connection with measuring the various string deflections on the tracing, a correction formula has been used to compensate for "high" or "low" calibration errors. The correction ratio which we employ uses the following factors:

Measured volume deflection : True volume deflection (X)
= Measured calibration deflection : 10

Thus if the measured volume deflection were 14 mm. and the standardization deflection were found to be 8 mm. instead of 10 mm. the measured deflection would be low and by the correction formula the true volume deflection would be:

$$14 : X = 8 : 10$$
$$8X = 140$$
$$X = 17.50 \text{ mm./5 c.c. of part (corrected deflection).}$$

Although this formula does not take into consideration all the variable factors which might cause minor errors, such as variation in volume of the trapped part, it is probably worth while in measuring volume pulses and blood flow where the standardization error is greater than 1 millimeter.

The illustrations demonstrate the essential details of this method of blood flow estimation in digits. The plethysmograms presented are tracings from normal individuals who have no evidence of peripheral vascular disease. The "warm room" flows have been recorded after one hour in an ambient temperature of 83° to 84° F. and the "cold room" flows after one hour in an ambient temperature of 68° to 69° F. (Fig. 3, *A* and *B*.)

The application of this type of digit blood flow determination to normal subjects and before and after limb sympathectomy will be presented in subsequent communications.

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*PROCEEDINGS OF THE CENTRAL SOCIETY FOR CLINICAL RESEARCH*

*Twenty-Second Annual Meeting*

*Chicago, Ill., Nov. 4 and 5, 1949*

## ABSTRACTS—Concluded

### 61. EXPERIMENTAL PRODUCTION OF MEGALOBLASTIC ANEMIA; AN INTERRELATIONSHIP BETWEEN ASCORBIC ACID AND PTEROYLGLUTAMIC ACID

CHARLES D. MAY, M.D., E. N. NELSON, M.D. (BY INVITATION), AND R. J. SALMON, M.Sc. (BY INVITATION), MINNEAPOLIS, MINN.

This report describes a means of producing megaloblastic and macronormoblastic anemia in monkeys using deficient diets alone.

The diets employed were basically dried cow's milk reconstituted with water and adjusted with lactose and vegetable oils and supplemented with crystalline vitamins to provide liquid diets of the following approximate composition:

| | PER 100 ML. |
|---|---|
| Protein | 1.6 Gm. |
| Carbohydrate | 7.2 Gm. |
| Fat | 3.3 Gm. |
| Calories | 67 |
| Vitamin A | 800 I. U. |
| Vitamin D | 50 I. U. |
| Thiamin | .07 mg. |
| Riboflavin | .1 mg. |
| Nicotinic acid | .5 mg. |

Any other vitamins present were those contained in the milk. Each experimental animal was given .137 mg. Fe per kilogram in the form of iron ascorbate intravenously at the start of the experiment followed by several weeks of oral therapy with 4 c.c. of ferrous gluconate daily.

In five control periods monkeys fed this type of diet ad libitum and 50 mg. ascorbic acid daily for five or more months remained in good health and did not develop anemia or changes in the bone marrow.

When the experimental diet was fed to eleven monkeys without the addition of ascorbic acid, a typical sequence of events occurred. For three to four months they remained in apparent good health. At about this time the first symptoms of scurvy appeared. They remained essentially in this state for the next three to four weeks, exhibiting only the symptoms of scurvy, without developing anemia or definitive changes in the marrow. Then a precipitous deterioration began. Pronounced anorexia and listlessness ushered in this phase. Diarrhea commenced invariably, the stools being loose to mushy, bulky, and foul but without pus or blood. With one exception a histamine refractory gastric achlorhydria developed. The fur lost its smoothness and luster and was shed in large amounts. The peridontal gingivae became ulcerated and necrotic. No changes were seen in the tongue. No infections were observed in the animals at any time. Anemia and neutropenia developed rapidly without evidence of additional hemorrhage. The anemia was usually of a normocytic or macrocytic type.

The most critical criteria were applied in designating the types of cells seen in the marrow. Three monkeys developed megaloblastic marrows very similar to, if not identical with, those seen in megaloblastic anemia in human

beings. The marrows in the other eight animals became macronormoblastic, a stage through which the megaloblastic marrows passed. The alterations in the granulocytes in each instance were striking, showing hypersegmentation and large early forms with premature lobulation such as are seen in the marrow in pernicious anemia. It was the impression that all the marrows might have progressed to a frankly megaloblastic picture if the precarious condition of the animals had not made it seem necessary to treat them in order to test the effects of various agents.

A systematic trial of vitamin $B_{12}$, PGA, and ascorbic acid revealed the following: (1) Ascorbic acid given relatively early in the disease gradually restored the animal to health and the marrow and blood became normal. (2) If ascorbic acid was not given until an advanced stage of the disease, it did not restore the marrow to normal or prevent the death of the animal. (3) Vitamin $B_{12}$ alone, given intramuscularly, had no effect on the marrow within forty-eight hours. (4) PGA, given orally or intramuscularly as the free acid or the triglutamate, but without ascorbic acid, promptly stimulated normal hematopoiesis in the marrow and relieved all the symptoms not attributable to scurvy. When ascorbic acid was added, normal health was restored even though the experimental diets were continued.

Increasing the protein in the diet to 3.3 Gm. per 100 c.c. by adding calcium caseinate did not prevent the development of the characteristic anemia.

Thus it would appear that megaloblastic anemia developed as a result of PGA deficiency somehow induced by a chronic deficiency of ascorbic acid.

## 62. CERTAIN EFFECTS OF CHEMOTHERAPY ON THE FECAL AEROBIC AND ANAEROBIC BACTERIA OF PATIENTS WITH CHRONIC ULCERATIVE COLITIS

HOMER C. MARSHALL, M.D. (BY INVITATION), WALTER L. PALMER, M.D., AND JOSEPH B. KIRSNER, M.D., CHICAGO, ILL.

The role of the fecal bacteria in the pathogenesis and course of chronic nonspecific ulcerative colitis is not clear. A method of simplifying the bacterial flora of the feces and reducing the number of bacteria in the bowel would provide useful information concerning this problem. Accordingly, qualitative and quantitative studies of the aerobic and anaerobic bacteria were made before and after treatment with absorbable and nonabsorbable sulfonamides, penicillin administered orally and parenterally, and the oral administration of streptomycin, aureomycin, and chloromycetin. Control studies consisted in observations intended to determine the error of the method and to ascertain the spontaneous day-to-day variation. The drugs were administered for relatively long periods of time; bacteriologic studies were carried out at frequent intervals in order to determine the initial and late effects of chemotherapy. All of the drugs, under certain conditions, are capable of altering the aerobic flora; some also may modify the anaerobic flora of the feces, the degree and duration of effect varying considerably. For instance, sulfonamides alter the aerobic flora, when initially used, for periods as long as six weeks. Streptomycin, on the other hand, exerts a marked quantitative effect, which is maintained, however, only for several days. Chloromycetin and aureomycin exert an intermediate effect: the *B. coli* disappear and the total bacterial count initially falls and then rises, with the reappearance of *B. coli* as a predominant organism. In addition, after the initial depression of bacterial counts had terminated, the counts tended to rise above the control levels and to remain high, decreasing to control levels only after the drugs were discontinued.

## 63. ALTERATIONS IN TESTICULAR STRUCTURE AND FUNCTION IN ORGANIC DISEASE OF THE PITUITARY

E. Perry McCullagh, M.D., Allen Gold (By Invitation), and J. B. Ralph McKendry (By Invitation), Cleveland, Ohio

A study was made of the gross and microscopic morphology of the testes in fourteen patients with organic pituitary disease. Changes in the gonads were correlated with prostatic size, urinary gonadotrophin and 17-ketosteroid excretion, and, when possible, with semen examination, the Kepler test, the adrenocorticotrophin test of Thorn, and the basal metabolic rate.

On gross examination the testes were found to vary from normal size and consistency to very small soft glands. Microscopically the picture varied from normal to complete loss of testicular architecture and extensive fibrosis.

Positive correlation was noted between testicular size and urinary F.S.H. excretion. In all cases in which the testes were normal in size the F.S.H. excretion was within the normal range, and, conversely, the finding of small testes was uniformly associated with a low urinary F.S.H. excretion.

Normal testicular size was usually associated with normal or nearly normal tubular structure but showed no constant relationship to the presence or absence of Leydig cells.

Testicular size correlated also with prostatic size in all but one case.

Histologically no degenerative changes were noted which would permit differentiation of primary testicular failure from that secondary to pituitary insufficiency.

## 64. NEUTROPENIA AND SPLENOMEGALY ASSOCIATED WITH RHEUMATOID ARTHRITIS

### Report of Four Cases With Splenectomy

Max M. Montgomery, M.D., and Robert L. Grissom, M.D., Chicago, Ill.

(Introduced by Louis R. Limarzi, M.D.)

During the past eight years four patients with the syndrome of neutropenia, splenomegaly, and rheumatoid arthritis have been studied. The rheumatoid arthritis in three patients was in remission and in the fourth was active. The ages ranged from 38 to 53 years. Three were women. They gave a history of rheumatic fever and presented findings of mitral stenosis. Arthritic symptoms had been present five, two and one-half, eleven, and twelve years, respectively. All had increased susceptibility to infections and low-grade pyrexia. None had skin pigmentation, lymphadenopathy, or leg ulcers.

The admission white blood cell counts were 1650, 2350, 1200, and 2100 per cubic millimeter, with 8, 33, 20, and 5 per cent neutrophils, respectively. All had hypochromic anemia but platelets were adequate. Sedimentation rates were elevated. Serum albumin /globulin values in Gm. per cent were 3.8/3.6, 3.3/2.1, 4.9/4.5, and 3.6/2.8, respectively. The bromsulfalein test and other liver function studies were normal. Bone marrow studies showed myeloid hyperplasia with maturation arrest at the myelocytic level.

These patients were given the diagnosis of Felty's syndrome, although they lacked lymphadenopathy and pigmentation. Three had rheumatic heart disease and all showed a low serum albumin and a tendency to approximation of A/G values.

Splenectomy was performed in all patients and by the third postoperative day the white blood cell counts were 10,250, 7,750, 10,000, and 9,700, respectively. The spleens showed follicular hyperplasia; the pulp was congested and exceedingly cellular and numerous phagocytic macrophages were present. Leucopenia has not been noted since operation. The anemia improved and serum albumin/globulin values became normal. The bone marrows no longer showed maturation arrest, and a shift to the right was manifest. All state they feel better, have comparatively few infections, and have no fever. Those with arthritis in remission have mild symptoms, and the fourth patient has improved markedly but additional treatment has probably been responsible.

These patients are living eight, four, two, and one year after splenectomy. We believe increased resistance to infection has enhanced their life expectancy. Splenectomy eliminated the leucopenia and anemia but had no definite effect on the arthritis. The association of rheumatic heart disease has been reported in only a few patients. These patients did not have subacute bacterial endocarditis. The low serum albumin values may reflect the nutritional status, incident to poor health, numerous infections, and weight loss rather than organic liver pathology.

## 65. QUANTITATIVE RESPONSE OF THE PROSTATIC ACID PHOSPHATASE OF THE IMMATURE RAT TO CHORIONIC GONADOTROPHIN AND EXTRACTS OF MALE URINE

E. Perry McCullagh, M.D., and C. A. Schaffenburg, M.D. (By Invitation), Cleveland, Ohio

The acid phosphatase was determined in the prostates of a group of intact immature white rats after the injection of graded doses of international standard chorionic gonadotrophin and the values were found to fall in a straight line. The curve thus obtained appears to be more sensitive than the weight-curve for seminal vesicles or prostate, and it is also responsive to the injection of male urinary extracts, thus promising to be of value in the quantitative evaluation of ICSH in normal and pathologic conditions.

Forty-eight hour urine specimens from normal male subjects were precipitated by the usual alcohol method; after purification by dialysis the final extract was taken up in 6.0 ml. of distilled water and injected subcutaneously into two twenty-one day old male rats, twice daily for two days and once on the morning of the third day. Rats injected either with standard preparations or with saline were used as controls. The animals were killed one hundred hours after the first injection and the prostatic acid phosphatase was determined by the method of Huggins and Talalay.

ICSH values for normal male subjects, expressed in terms of gamma of international standard chorionic gonadotrophin, have been found to run between 100 to 200 gamma per twenty-four hours.

Work is now in progress to determine the clinical value of the test as well as its specificity.

## 66. A COMPARISON OF DIFFERENT REGIMENS IN THE TREATMENT OF HEPATIC CIRRHOSIS

Gordon R. Morey, M.D. (By Invitation), and Robert M. Kark, D.C.H., Chicago, Ill.

Over the past two years, clinical, laboratory, and metabolic observations were made on patients with hepatic cirrhosis during treatment on different therapeutic regimens.

All of the patients improved when a basic regimen was instituted which provided a diet which contained calories equivalent to twice the calculated basal requirement; 2½ Gm. of protein per kilogram of body weight; 30 per cent of the calories as fat and the remainder as carbohydrate. The patients' clinical improvement was accelerated by the addition, to this regimen, of daily intravenous infusions of a mixed amino acid solution which was sodium free.

Restriction of the sodium intake below 1.0 Gm. per day was a valuable adjuvant in combating fluid retention. The patients with edema and ascites showed an increased urinary output and the edema and accumulation of ascites diminished when salt intake was restricted.

No clinical benefits were observed in the patients when the basic regimen was supplemented with cystine, methionine, choline, B complex vitamins, or parenteral liver.

The infusion of large amounts of concentrated human serum albumin (salt poor) was impractical as a method of protein supplementation, even though a transient rise in serum albumin concentration and a marked increase in positive nitrogen balance attended its administration.

On the regimen described (diet, salt restriction, and supplementation with amino acid infusions) all of the patients showed increased weight gains without fluid retention; those with edema and ascites gradually showed reduction and disappearance of these manifestations of liver disease. All patients showed a striking capacity for the retention of tremendous amounts of nitrogen, probably due to replacement of fluid in the tissues by newly synthesized muscle protein.

One patient with advanced cirrhosis and recurrent ascites was observed over a period of two years while subsisting on seven different regimens. At the peak of the patient's illness, paracenteses were necessary at ten to twenty-day intervals and 16 to 19 liters of fluid were removed at a time. Albumin therapy produced no alteration in the rate of accumulation. A low salt regimen reduced the frequency of paracenteses to about thirty-day intervals. Finally, with the previously described regimen, the patient became ascites free and has remained so for 7 months. He has maintained his body weight gains and has returned to a normal active existence.

Of seven liver function tests, serum cholinesterase activity was the most reliable test of liver function in that the cholinesterase levels most accurately correlated with the patient's clinical condition.

## 67. QUANTITATIVE STUDIES OF VIBRATORY PERCEPTION IN DIABETIC AND NONDIABETIC SUBJECTS

I. Arthur Mirsky, M.D., Perry Futterman, M.D. (By Invitation), and Robert H. Broh-Kahn, M.D., Cincinnati, Ohio

Quantitative measurements of the threshold of vibratory perception were made in 102 diabetic patients and in 136 nondiabetic, nonobese, nonhypertensive subjects. Both groups of patients were chosen at random from an ambulatory population and were from 5 to 79 years of age.

No correlation was found to exist between the threshold of vibratory perception and the side of the body tested or the sex of the subject. A significant increase in the threshold was found in the diabetic group of subjects. This increase was not related to either the known duration of diabetes mellitus or the severity of the metabolic derangement as estimated by the patients' insulin requirements. Diabetic subjects with symptoms or signs of neuropathy tended to have a greater incidence and greater degree of vibratory

impairment than those without such signs or symptoms. Similar measurements in nondiabetic groups of obese and hypertensive patients revealed no differences in their thresholds from those of the control group.

Both the diabetic and nondiabetic groups of subjects showed an increasing threshold of vibratory perception with increasing age. This increase was more marked in the toes than in the fingers and was greater in the diabetic than in the nondiabetic subjects. The data revealed a more rapid increase in vibratory threshold among the diabetic subjects during the earlier decades; during the later years of life, the threshold of the nondiabetic subjects rapidly approached the level of the diabetic subject.

This study provides evidence for the concept that the process of aging is more rapid, or of greater initial intensity, in the patient with diabetes mellitus than in the nondiabetic subject.

## 68. MECHANISM OF HYPERTHERMIA NOT DUE TO INFECTION

MAX M. MONTGOMERY, M.D. (BY INVITATION), FORD K. HICK, M.D., AND ROBERT WOOD KEETON, M.D., CHICAGO, ILL.

Fever usually indicates infection but also occurs when an individual cannot eliminate heat produced by his body metabolism, a principle utilized in fever production by the various "cabinets."

In the past few years a number of patients have been observed with fever not easily ascribed to infection. Records of nine such patients permitted study of the rectal temperature, state of the skin surface, pulse rate and rhythm, blood pressure, and environmental conditions. Six developed fever ranging from 104 to 107.8° F. during hot humid weather, and three developed fever ranging from 102.8 to 106° F. during moderate weather. Six were postoperative and 3 were medical patients.

Failure of transport of heat from the interior to the body surface with consequent heat retention was indicated in five of the postoperative and two of the medical patients by a cold skin and a high rectal temperature. In each case a circulatory factor was present; auricular fibrillation in two, excessive tachycardia in two, severe hemorrhage in one, shock following myocardial infarction in one, and circulatory collapse in acute liver necrosis in one.

Failure to eliminate heat from a hot skin was present in one patient with ichthyosis who was virtually unable to sweat and in whom anesthesia, surgery, and excessive bed covering combined with very hot weather to produce a rectal temperature of 105° F. An overdose of barbiturate led to hyperthermia of 104.6° F. in one patient found unconscious in a hot humid room. Dehydration, pulmonary edema, and anoxia plus a disturbance of the heat regulatory mechanism were contributory factors.

In each patient several factors were operative, but the common factor was the inability to lose heat as rapidly as it was produced, usually due to inadequate circulation. A hot humid environment was often the precipitating factor since this interfered with heat loss. The load on the cardiovascular system was increased by heat retention and attendant tachycardia. This led to excessive tachycardia or auricular fibrillation with less efficient circulation and further heat retention.

From the study of these patients with severe hyperthermia one is led to consider the factor of heat retention in fevers of lesser degree such as occur in congestive heart failure, shock, myocardial infarction, etc. Measures aimed at facilitating heat loss at the inception of fever may prevent the serious effects of hyperthermia.

## 69. THE EFFECT OF HIGH VAGUS SECTION UPON THE CLINICAL PHYSIOLOGY OF THE BRONCHI

DOUGLAS R. MORTON, M.D. (BY INVITATION), KARL P. KLASSEN, M.D. (BY INVITATION), AND GEORGE M. CURTIS, M.D., COLUMBUS, OHIO

Other than the occurrence of substernal pain in patients having acute bronchitis, little is known concerning pain of bronchial origin. Consequently, an evaluation of the quality as well as the referral of pain of bronchial origin was made in patients by direct stimulation of the bronchial mucosa, using an electrode introduced through a bronchoscope. Following recovery from the bronchoscopy, the patients described the sensation as a moderately intense aching pain, which was felt in the homolateral anterior chest or the homolateral anterior cervical region. In a series of twenty-five patients thus investigated, the referral of pain was consistent and symmetrical.

Patients found to have inoperable bronchogenic carcinoma at exploratory thoracotomy afforded an opportunity to evaluate the effect of vagotomy on the clinical physiology of the bronchi. Accordingly, in these patients the vagus nerve was transected above the pulmonary plexus, yet below the origin of the recurrent laryngeal nerve, with the following results:

(1) The *cough reflex* arising from the homolateral bronchial tree was abolished in all instances. (2) In the majority of cases *pain* of bronchial origin was abolished on the homolateral side. In the remaining few, there was referral of pain to the contralateral anterior cervical region. (3) No effect on the physiologic respiratory change in *bronchial caliber* was noted, nor was any effect observed on *bronchial motility*. (4) On bronchoscopic observation no subsequent changes were noted in the amount nor in the consistency of *bronchial secretions;* likewise, postbronchography roentgenograms revealed no impairment of Lipiodol clearance of the tracheobronchial tree. (5) In a limited number of cases, electrocardiographic tracings were unaffected by compression of the vagus nerve or its transection or by electric stimulation of either the proximal or distal ends of the transected vagus.

## 70. PYRIDOXINE DEFICIENCY IN HUMAN BEINGS INDUCED WITH DESOXYPYRIDOXINE

JOHN F. MUELLER, M.D. (BY INVITATION), AND RICHARD W. VILTER, M.D., CINCINNATI, OHIO

Since 1934, when György announced the existence of a new vitamin for the rat, which when synthesized was called pyridoxine, numerous reports have appeared clearly defining deficiency states in various laboratory animals. These have included seborrhea-like dermatitis, muscular weakness, decrease in lymphoid tissues, and decrease in circulating antibodies in the rat, hypochromic anemia in the dog, anemia and central nervous system lesions in swine, and lymphocytopenia and absolute increase in neutrophiles in the monkey. There has never been a clear-cut demonstration that pyridoxine is essential to human beings and a pyridoxine deficiency state in man is unknown.

With the recent advent of metabolite inhibitors, a new tool has been placed in the hand of the investigator to study deficiency states. Such antagonistic activity toward pyridoxine has been demonstrated for desoxypyridoxine in the chick. During pilot experiments to determine the toxicity of desoxypyridoxine in man, we observed a deficiency syndrome precipitated

by a vitamin B complex-poor diet and desoxypyridoxine which responded specifically to pyridoxine. These early experiments have been extended and are the basis of this report.

Eight patients suffering from various chronic illnesses were given 60-150 mg. of desoxypyridoxine intramuscularly daily while they were maintained on a diet low in vitamins of the B complex. Hematologic studies were done before and at biweekly intervals after the institution of the experimental regime. Urine assays for pyridoxic acid, thiamin, riboflavin, and $N^1$-methylnicotinamide were done at weekly intervals in most instances. When the deficiency state developed, the patient was placed on a vitamin B complex mixture devoid of pyridoxine and later on pyridoxine alone. The desoxypyridoxine and diet were continued throughout the entire experimental period.

Seborrhea-like skin lesions developed about the eyes, nose, and mouth within two to three weeks in seven of the eight patients. In three of the seven the lesions were quite severe. Half of the patients developed erosions in and around the mouth resembling cheilosis of riboflavin deficiency and glossitis resembling morphologically that seen in niacin deficiency. One patient developed severe systemic symptoms. A mild but definite lymphocytopenia appeared in seven of the eight patients. No anemia occurred. These manifestations were unchanged when the vitamin B complex mixture devoid of pyridoxine was given but responded in forty-eight to seventy-two hours to the parenteral administration of 100 to 200 mg. of pyridoxine.

The authors feel that these lesions constitute at least one part of the human syndrome of acute pyridoxine deprivation.

## 71. PANCREATIC DYSFUNCTION AND LIVER DISEASE

R. O. Muether, M.D., William Knight, Jr., M.D. (By Invitation), and Vincente Moragues, M.D. (By Invitation), St. Louis, Mo.

Advances have been made in the knowledge of the etiology and pathogenesis of liver and pancreatic diseases. Recent animal studies by Chaikoff, Best, Gillman, Ivy, and others have shown that fatty deposition in the liver or fibrosis may follow inflammatory changes in the pancreas, pancreatic duct ligation, and pancreatectomy in dogs maintained on a diet of lean meat and insulin. The hepatic changes could be prevented by the feeding of pancreatic tissue and lipotrophic agents.

Experimentally there appears to be sufficient evidence to indicate that disturbance in pancreatic function may lead to serious liver disease.

Hepatic changes have been demonstrated to accompany certain pancreatic diseases such as diabetes mellitus and acute pancreatitis. However, pancreatic dysfunction as a cause of serious permanent liver damage has largely been overlooked.

The use of frequent serum diastase determinations and the prostigmine diastase test has increased the accuracy and incidence in the diagnosis of pancreatic dysfunction and has enabled us to detect abnormalities of the pancreas when diseases of other organs in the upper abdomen may be also present.

We wish to report the case histories and laboratory data of five patients who had pancreatic disease as shown by the clinical history, elevated random diastase determinations, and abnormal prostigmine diastase tests and who also had hepatic disease. These diagnoses were confirmed by surgical exploration and biopsy.

The pathology in the liver ranged from periportal round-cell infiltration, similar to that seen in cholangiolytic hepatitis, to the marked fibrosis of advanced hepatic cirrhosis.

In none of the cases could the usual etiological agents of liver disease such as toxins, infection, or malnutrition be elicited. The elevated serum diastase values which occurred at intervals in these patients as well as the abnormal prostigmine diastase test are at variance with the low serum diastase values usually found in patients with damaged livers. These findings and the experimental work of Chaikoff and others, which indicate that removal or damage of the pancreas in dogs and rats will result in fatty infiltration of the liver, as well as inflammatory changes and various types of fibrosis, lead us to suspect that pancreatic dysfunction may at times play an important etiological role in the pathogenesis of liver disease.

## 72. CHANGES OBSERVED FOLLOWING THE EXPERIMENTAL INFUSION OF THE DIURETIC SODIUM SULFATE

L. A. Nalefski, M.D., Chicago, Ill.

(Introduced by N. C. Gilbert, M.D.)

The diuretic ability of sodium sulfate was established some sixty years ago in experimental animals. It was first administered parenterally to human subjects in 1934. No adverse effects were reported in normal subjects receiving moderate doses, and when given to patients with diminished urine outputs, it appeared to be nontoxic. Maitland encouraged its use for anuria resulting from crush injuries during the "blitz" in England, but it was not widely adopted. Recently the Council on Pharmacy and Chemistry of the American Medical Association granted its approval for the parenteral use of sodium sulfate as a diuretic in the treatment of oliguria or anuria secondary to the crush syndrome, burns, transfusion reactions, toxic hemolysis, and obstructive lesions of the genitourinary tract.

The author observed the use of this diuretic in four patients. In two with cardiac edema, one responded well and the other expired suddenly several hours after 1 liter of isotonic sodium sulfate was administered. Two patients with anuria resulting from hemolytic reactions consequent to receiving improperly typed blood expired without diuresing. Unusually high blood sulfate levels were found following the use of sodium sulfate.

The foregoing clinical observations prompted further experimental study of this drug. Healthy female dogs were anesthetized, after which they were infused with isotonic sodium sulfate. A control group of animals was studied using isotonic sodium chloride. The infusions were maintained for a period of five hours. At hourly intervals arterial blood samples were obtained and subjected to a complete electrolyte and acid-base study. Urine was collected quantitatively during the same intervals and studied similarly. Electrocardiographic tracings were made initially and at the end of each hour. At the end of the five-hour infusion the animals were sacrificed and tissue biopsies obtained.

It was found that a true acidosis developed following sodium sulfate infusion and that this acidosis could be mitigated by the proper use of buffers in the infusion media. Histologic changes were present in the kidneys of those animals infused with sodium sulfate and absent in the control group. Electrocardiographic changes were also demonstrated during the sodium sulfate infusion.

It is concluded that the indiscriminate use of sodium sulfate as a diuretic in cases of anuria is dangerous. By utilizing a buffered solution with the sodium sulfate, it is thought that some of this danger may be eliminated.

## 73. CARDIOVASCULAR CHANGES FOLLOWING THE EXPERIMENTAL ADMINISTRATION OF BARIUM CHLORIDE

L. A. Nalefski, M.D. (By Invitation), N. C. Gilbert, M.D., and G. K. Fenn, M.D., Chicago, Ill.

During recent experiments with various heparin preparations it was found that the sodium salts of heparin increased the coronary flow volume, while the barium salts of heparin did not show this effect. In earlier work in coronary flow studies we gained the impression that the soluble barium salts acted as a coronary constricting agent. We therefore decided to study the action of this drug on the cardiovascular system in detail.

Healthy mongrel dogs, ranging in weight from 10 to 12 kilograms, were given barium chloride orally and parenterally in a conscious state. Electrocardiographic and plethysmographic tracings were made. Blood pressures were recorded. The effect on coronary flow was determined in anesthetized animals by cannulating the coronary sinus and also by studies on the empty beating heart.

Barium chloride administration was followed (a) by electrocardiographic changes such as seen in coronary insufficiency and (b) by a variety of arrhythmias. A hypertensive effect was produced with a slowing of the heart rate. The plethysmographic tracings indicated a generalized vasoconstrictor effect. Coronary flow was markedly decreased in the experiments conducted.

There appeared to be a difference in tolerance of the drug in various animals. Some would suddenly expire with agonizing cries, and electrocardiographic tracings in these animals indicated that death was caused by coronary insufficiency. Autopsies made immediately after death showed grossly areas which were similar in appearance to those found immediately following ligation of a coronary artery. All of these toxic effects of barium chloride could be delayed or prevented by treating the animal previously or during the experiment with aminophyllin or papaverine.

It has been shown by others that barium chloride is precipitated within a matter of several minutes by the sulfate ions normally present in the blood stream. Therefore, it was difficult to explain the prolonged effects that barium chloride administration sometimes exhibits. Because of this paradox, colloidal barium sulfate preparations were made and given parenterally. Similar electrocardiographic changes were obtained with this molecular preparation as with the ionized salts.

From these experiments we conclude that the clinical use of barium chloride in the Stokes-Adams syndrome, or for any other purpose, is dangerous and unpredictable.

## 74. KIDNEY EXCRETION DURING AND AFTER HEMOGLOBINEMIA

### A Method for Production of Hemoglobinemia by High Sonic Vibration

William H. Olson, M.D. (By Invitation), and H. Necheles, M.D., Chicago, Ill.

Our goal in this work was to obtain an experimental animal (dog) with complete suppression of kidney excretion due to hemoglobinemia, the animal to remain anuric and die in uremia from five to ten days after the onset of hemoglobinemia. Acute and chronic experiments were done on large, healthy, male dogs.

Acute experiments were performed using the following methods for producing hemolysis: thermal trauma, intravenous injection of distilled water, intravenous injection of hemoglobin solution, intravenous injection of hemolytic agents, heating and cooling blood, and, finally, use of high frequency sonic vibrations. The latter method proved to be the most useful, and it was employed throughout most experiments. Blood pressure, urine excretion, blood studies, and survival time were studied. For production of the sonic hemolysis, a magnetostriction oscillator was used that produced high energy by giving off vibrations in the region of high sonic frequency. The magneto-oscillating rod was placed into a glass cup with two side cannulas which were connected to blood vessels. The blood was hemolyzed as it flowed past the rod. An extremely high degree of hemoglobinemia could be obtained; the highest value was 11,125 mg. per cent of plasma hemoglobin.

In well-hydrated animals with no hemoconcentration, hemoglobinemia may produce complete suppression of kidney excretion during the period of hemolysis. Following this the animal may develop diuresis when plasma hemoglobin is below 5,000 mg. per cent; oliguria when plasma hemoglobin is between 5,000 and 8,000 mg. per cent; or anuria when plasma hemoglobin is above 8,000 mg. per cent. In an animal with moderate reduction of blood pressure, together with a progressing hemoconcentration, the production of hemoglobinemia with levels above 2,500 mg. per cent resulted in anuria.

The duration of the period in which hemolysis was produced varied between ten and sixty minutes. It is felt that, for the study of experimental anuria, rapid hemolysis must be used, in order to obtain comparable experimental animals.

## 75. CARDIAC FACTORS IN "NEUROGENIC" PULMONARY EDEMA

ROBERT PAINE, M.D. (BY INVITATION), HARVEY R. BUTCHER, M.D. (BY INVITATION), AND JOHN R. SMITH, M.D., ST. LOUIS, MO.

Experimental evidence indicates that Starling's principles of fluid balance are applicable to the lung. Pulmonary edema follows either a diminution of blood osmotic tension or an increase in capillary hydrostatic pressure. Furthermore, increased pulmonary hydrostatic pressure, sufficient to provoke edema, results when cardiac overload is unequally thrown upon the left ventricle. It is likewise possible that left ventricular overload may be produced by "neurogenic" factors. Increased intracranial pressure or vagal stimulation are reported to cause pulmonary congestion and edema through cardiovascular responses extraneous to the lungs.

In open-chest preparations of dogs, pulmonary and systemic arterial and venous pressures were simultaneously recorded. The administration of epinephrine (0.25 to 1.0 mg. intravenously) produced the usual striking rise of arterial pressure and marked bradycardia. Cardiac dilatation and elevation of pulmonary venous and arterial tensions occurred, with congestion and edema of the lungs. Conversely, when bilateral faradic vagal stimulation was applied, the resulting bradycardia was accompanied by a fall of systemic arterial pressure; pulmonary congestion and edema did not occur. It is suggested that sufficient elevation of systemic arterial pressure, together with critical bradycardia (reduced minute volume output), from epinephrine, may overload the left ventricle *if* there is continued competence of the right ventricle and optimum venous return. The experiments suggest that neurogenic influences may alter the pulmonary vascular dynamics through adjustments that lead to impaired left ventricular function.

## 76. DIABETES DETECTION

BRUNO J. PETERS, M.D., MILWAUKEE, WIS.

(INTRODUCED BY MAURICE HARDGROVE, M.D.)

Diabetes detection is becoming a problem of increasing importanee. Early diagnosis may be the only way one can hope to modify or possibly cure the disease. This is a preliminary report on a diabetes detection survey started in September, 1948, which is still being carried on at an industrial plant in Milwaukee, Wis. To date, 180 patients have been tested, 69 female and 112 male, their ages ranging from 18 to 75 years. The patients are an unselected group who come to the medical department for routine physical examinations or come with minor ailments. No patient with a definite history of diabetes or symptoms suggestive of diabetes is included in the present report. The individual, besides having routine laboratory examinations, was given 100 Gm. of glucose on a fasting stomach and a blood sugar determination two hours after the ingestion of the glucose. The blood sugar determinations were done by the Folin-Wu method; recently the Somogyi method has been used in addition.

If the blood sugar report was 130 mg. or over, the patients were told to eat a normal diet during the next three days and warned against missing meals or fasting. After that period a glucose tolerance test was done. In those patients who fulfilled the following criteria, presence of sugar in the urine together with peak level above 170 mg. per cent (Folin-Wu) after the ingestion of glucose, a diagnosis of diabetes was made. Eleven cases fell in this group.

Five patients were diagnosed as having a prediabetic state, because these patients demonstrated diabetic glucose tolerance curves but did not have sugar in the urine. A diabetic curve is one with a peak level above 170 mg. per cent and a level above 130 mg. per cent two hours after ingestion of 100 Gm. of glucose. In these five patients the peak levels ranged from 196 to 216, and none had a return of B.S. to below 132 in two hours. Two patients were classed as potential diabetic patients. They had peak levels of 225 and 187, respectively, and two-hour levels of 108 and 114 with no sugar in the urine.

On the basis of this survey, eleven patients, or 5.1 per cent, had definite diabetes, as proved by an abnormal glucose tolerance and sugar in the urine. Five patients (2.8 per cent) had abnormal glucose tolerance curves but had no sugar in the urine, and two (1.1 per cent) were classified as potential diabetic patients. None of these patients have symptoms of diabetes. Some have had several determinations during the year and continue to show the same abnormal tolerance curves. Only seven of these patients have fasting blood sugars above 120 mg. per cent, the highest being 151 mg. per cent.

If one considers the first two groups collectively, approximately 7.9 per cent of the patients will have diabetes. It is felt that screening methods for diabetes will uncover a large number of diabetic patients, but many will still be missed unless more accurate testing is done.

## 77. INTERPRETATION OF THE RESULTS OF THE FLOCCULATION TESTS ON BASIS OF BIOPSY FINDINGS AND PROTEIN PARTITION

HANS POPPER, M.D., FREDERICK STEIGMANN, M.D., J. DE LA HUERGA, M.D. (BY INVITATION), CHICAGO, ILL., AND MURRAY FRANKLIN, M.D. (BY INVITATION), IOWA CITY, IOWA

The diagnostic significance of the flocculation tests in hepatobiliary diseases is not fully understood despite their established practical value. Therefore, in 187 cases with various liver diseases (226 series of determinations), the results of cephalin flocculation (CF), thymol turbidity (TT), zinc sulfate turbidity (ZST), and the recently described gamma globulin turbidity (GGT) were compared with those of chemical and electrophoretic partition of serum proteins into albumin and the various globulins and, where available, with the findings in liver biopsy specimens. This correlation indicated the following factors as influencing the results of the flocculation tests.

1. Reduction of serum albumin, mostly as result of liver cell damage. This increased all flocculation tests except the GGT.

2. Elevation of gamma globulins in hepatic disorders mostly related to proliferation of Kupffer cells and other mesenchymal cells and histologically recognized by appearance of cytoplasmic ribonucleic acid. This is possibly an expression of antibody formation or of stimulation by liver cell breakdown products. This factor tends to elevate all flocculation tests. It also explains the elevation of GGT and ZST in recovering viral hepatitis, being more marked with tendency for cirrhosis formation.

3. Depression of gamma globulin formation in severe jaundice as result of intra- or extrahepatic biliary obstruction, apparently related to excessive bile pigment imbibition of the Kupffer cells.

4. Depression of ZST but also CF and TT (despite elevation of the gamma globulins) by a humoral factor occurring in obstructive jaundice (probably of biliary nature) and occasionally in cirrhosis without jaundice (so far of unknown character).

5. A factor probably resulting from qualitative changes of serum albumin producing elevation of CF and TT, not explained by changes in the albumin/gamma globulin ratio.

6. Lack of a TT enhancing factor, possibly of lipid character but not related to total lipid or cholesterol concentration. This accounts for the often normal or only slightly increased TT in alcoholic cirrhosis.

Interplay of these factors produces diagnostic patterns of the flocculation tests in most conditions. Only in toxic hepatitis was the pattern not characteristic. The third and fourth factors account for the normal flocculation tests in extrahepatic biliary obstruction and occasionally in hepatitis with intrahepatic obstruction, both even in presence of severe liver damage. Superimposed bacterial infection in extrahepatic obstruction may raise GGT, CF, and TT without necessarily elevating ZST. Understanding of these factors and the performance of all four flocculation tests increases their diagnostic value.

## 78. A PRELIMINARY REPORT ON THE STUDY OF MYOCARDIAL INFARCTION BY AURICULAR CATHETERIZATION

WALTER H. PRITCHARD, M.D., HERMAN HELLERSTEIN, M.D. (BY INVITATION), ROBERT LEWIS, M.D. (BY INVITATION), AND SCOTT INKLEY, M.D. (BY INVITATION), CLEVELAND, OHIO

In a preliminary experience initiating a study of the dynamic effects of acute myocardial infarction, six patients were studied by the right auricular catheterization technique. All patients had definite evidence of the onset of an acute infarct both from clinical and electrocardiographic studies ten to seventeen days prior to catheterization. Cardiac rhythm was normal and congestive failure absent. This postinfarction period was selected as the period of initial study because of the theoretical hazards accompanying catheterization immediately following an acute infarct. After sufficient data and experience have been acquired in the study of this period, observation will be extended to the more immediate postinfarction period.

In nine control patients hospitalized for a similar period of time with diseases other than cardiac disorders, cardiac indices ranged from 2.8 to 3.8 liters per minute per square meter of body surface.

No difficulties or complications have been encountered during or after catheterization. Basal oxygen consumption and right auricular pressures during the procedure were within normal limits in all patients.

In three patients, cardiac output, arterial blood pressure, and heart work were within normal limits. In the remaining three patients, all of whom had low blood pressures, work was reduced approximately 30 per cent below the lower normal range of the control values.

One patient was catheterized on the tenth and again on the thirtieth postinfarction day. At the first catheterization values for output and work were low. Following the usual clinical improvement during the following three weeks, blood pressure and work rose, but cardiac output remained unchanged.

These studies are of a preliminary nature and no conclusions relative to factors leading to reduced output or maintenance of a normal output following infarction can be drawn. In the present small series of cases, there was no correlation between the apparent size of the infarct determined from the electrocardiogram and the level of cardiac output.

## 79. TWO RARE CASES OF CONGENITAL MALFORMATION OF THE HEART OF THE CYANOTIC GROUP; RIGHT HEART CATHETERIZATION AND ANGIOCARDIOGRAPHIC STUDIES

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Two cases of rare anomalies of the cyanotic group which must be differentiated from the tetralogy of Fallot are presented.

The first case is that of a girl 5½ years of age with symptoms of mild dyspnea and cyanosis on exertion. Cardiac catheterization and anigocardiography demonstrate a complex malformation consisting of (a) a single ventricle, (b) a pseudotruncus arteriosus with probable hypoplasia of the right pulmonary artery, and (c) aberrant venous coronary drainage directly

into the common ventricle. Data and pressure tracings are presented. Differential diagnosis and certain hemodynamic factors, particularly those which may disturb the balance between the systemic and pulmonary circuits, are discussed.

The second case is that of a 4-year-old girl with persistent cyanosis. Data obtained by right heart catheterization reveal (a) transposition of great vessels, (b) intraventricular septal defect, and (c) patent ductus arteriosus. The problem of differential diagnosis in this case between the Eisenmenger complex and Taussig's heart, consisting of transposition of the aorta and overriding pulmonary artery, is outlined and the dynamics are discussed.

## 80. STUDIES ON THE SPREAD OF EXCITATION THROUGH THE VENTRICULAR MYOCARDIUM

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Direct lead electrocardiograms have been recorded on isolated perfused dogs' hearts subjected to certain injuries designed to influence the spread of the excitatory process through the ventricular myocardium. These injuries were produced by the introduction into the left or right ventricular cavity of solutions or crystals of potassium chloride, silver nitrate, phenol and cocaine.

Injuries sufficiently severe to be attended by the electrocardiographic pictures of bundle branch block did not evoke any specific effect which might be ascribed to arborization block consequent to destruction of Purkinje fibers. As complexes typical of bundle branch block developed in epicardial leads, the QRS complex in leads from the ventricular cavity into which the traumatizing agent had been introduced assumed an essentially monophasic character, with an R wave very similar to that recorded simultaneously from a contiguous epicardial lead. Apparently, endocardial and epicardial electrodes bore a similar spatial relationship to the spread of excitation through the intervening ventricular wall.

In an attempt to account for these findings, the following hypothesis was proposed. The speed at which the excitatory process spreads through a certain segment of the myocardial syncytium depends upon the orientation of the fibers in that segment to each other and to the point of origin of excitation. Spread down a strip of fibers of which the long axis is parallel to the long axis of the strip is rapid as compared with spread down a strip in which the fibers run at a right angle to the long axis of the strip. Endocardial activation is rapid not because of the presence of Purkinje fibers, but because the subendocardial bands of myocardium form a network through which excitation can move rapidly along the long axis of the fibers. Spread across the septum in bundle branch block and across the free wall of the left ventricle in the normally activated heart is slow because excitation is moving through fibers, the long axis of which is perpendicular to the advancing wave of excitation.

Testing of this hypothesis was undertaken by isolation of a segment of the ventricular wall, except for a limited attachment at one end of the strip. The spread of excitation down this segment was studied in relation to the disposition of the fibers constituting the strip. The presence of a band of fibers, the long axis of which is parallel to the long axis of the strip, appears to be essential to rapid excitation of the segment. Whether these fibers are epicardial or endocardial makes no difference.

## 81. THE PATHOLOGIC PHYSIOLOGY OF MEGA-ESOPHAGUS

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The actual underlying physiopathology of mega-esophagus is still a matter of uncertainty, in spite of many theories set forth. Because methods of determining the functional behavior of various parts of the esophagus have heretofore been so equivocal and inadequate, we have felt that esophageal motility studies by the balloon and kymograph method might aid in solving this problem. We have made extensive esophageal motility studies under varying conditions of three normal persons, three patients with achalasia, and five patients with organic obstructive lesions of the esophagus.

The three normal persons served as controls. They were studied under varying conditions over many hours. These studies show that organized frequent contractions of good amplitude are related normally with powerful peristaltic waves and an excellent deglutition effect, as revealed by a strong pull on the Einhorn tube attached to the balloon. They play an outstanding role in Magendie's third stage of swallowing.

Information obtained by fluoroscopy and x-ray film studies during barium swallow and by observed clinical effects on the inflated balloon which acted as a bolus of food was complementary to that obtained from the esophageal motility studies. The data considered together gave adequate conceptions of the action of the musculature of the esophagus. These data indicated that the intrinsic motility of mega-esophagus varies greatly, being almost always irregular, uncoordinated, hyporesponsive to stimuli, and not completely effective. In fact, the intrinsic motility at times completely failed, and in one patient was entirely absent, indicating total motor paralysis. This differs from that of the organic obstructive lesions of the esophagus in which there usually occurs a normal motility in the segment proximal to the lesion, but rarely a hypermotility or even a hypomotility, depending upon the degree of obstruction and the stage and complications of the organic disease. Achalasia of the esophagus also differs from true esophagospasm in which there usually occurs a hypermotility and hyperirritability.

These data indicate that the disease in idiopathic mega-esophagus is not localized necessarily to the cardiac end of the esophagus as is commonly believed. In our experience functional abnormalities often involve the entire length of the esophagus. These newer observations offer further evidence for, and are compatible with, the general theory of achalasia as an underlying mechanism of cause of idiopathic mega-esophagus.

A discussion will be presented of the effects of atropine and sympathicolytic and parasympathomimetic drugs upon the disturbed peristalsis. The clinical implications of these data will be set forth.

## 82. THE PROTHROMBIN ACTIVITY OF HUMAN BLOOD

Armand J. Quick, M.D., and Mario Stefanini, M.D. (By Invitation), Milwaukee, Wis.

In 1943, when the labile factor was discovered, it was noted that the addition of a small amount of deprothrombinized rabbit plasma, which has a high concentration of labile factor, to stored plasma reduced the prothrombin time markedly below normal. Recently it was found that if blood was carefully collected and stored in silicone-coated glassware, the prothrombin activity decreased on storage, but the addition of deprothrombinized rabbit plasma, that is, excess labile factor, reduced the prothrombin time only to the

level of fresh plasma. By means of the direct method of determining prothrombin by adsorption with tricalcium phosphate and elution with sodium citrate, it was further found that plasma stored in glass twenty-four hours or longer contained a much greater quantity of prothrombin than fresh or stored plasma kept in a silicone-coated container.

Obviously prothrombin activity increases in plasma stored in glass. This is masked by the decrease in labile factor and only becomes evident when labile factor is readded. In a silicone-coated container such an augmentation does not occur during several days of storage. The change in prothrombin activity is explained by postulating that prothrombin in circulating blood exists both in an active and in a precursor state. In contact with a rough surface such as glass the precursor is converted to the active form. In oxalated plasma the activation is relatively slow, while in native plasma it occurs in less than an hour. What factor other than the surface of the container is necessary for this conversion to active prothrombin remains undetermined. Platelets, calcium, and thrombin are not essential for this reaction.

Total and free prothrombin and the conversion to the active form are normal in hemophilia and thrombocytopenic purpura. In Dicumarol poisoning both active and total prothrombin are reduced. In one type of congenital hypoprothrombinemia both the active and total prothrombin are below normal; in a second type the active is low but the total may be normal.

In summary, prothrombin in human blood occurs partly in an active form and partly in a precursor state. Prothrombin activity as measured by the prothrombin time is dependent upon the concentrations of (1) active prothrombin (not total), (2) labile factor, and (3) bound calcium. Whether the inactive prothrombin acts as a reserve or becomes activated during standing is not known.

## 83. THE EOSINOPHIL RESPONSE IN ADRENOCORTICOTROPIC HORMONE (ACTH) THERAPY

THERON G. RANDOLPH, M.D., DAVID E. MARKSON, M.D. (BY INVITATION), AND JOHN P. ROLLINS, M.D. (BY INVITATION), CHICAGO, ILL.

Ten patients with diagnoses of rheumatoid arthritis, ulcerative colitis, bronchial asthma, and other allergic disturbances were observed prior to, during, and, in some instances, following the cessation of therapy with ACTH-Armour by means of serial direct counting chamber eosinophil determinations of the peripheral blood with propylene glycol-phloxine stain diluting fluid as previously described by one of us (T. G. R.).

An average of eleven blood eosinophil determinations was made per patient prior to starting ACTH therapy. The average number of circulating eosinophils was found to be 472, the range varying from a minimum of 55 cells to a maximum of 2,354 cells per cubic millimeter of blood.

Eosinophils disappeared from the peripheral circulation following the intramuscular administration of ACTH in all instances, confirming the observations of Hills, Forsham, and Finch. An absence of circulating eosinophils was first noted in one case at six hours and in another at seventy-two hours, with an average of twenty-eight hours. The dosage of ACTH ranged from 40 to 200 mg. per day. The total quantity required for this eosinophil response varied from 15 mg. in one indivdiual to 400 in another, averaging 112 mg. per twenty-four hours.

One patient was treated with 100 mg. daily for seventy-eight hours, two for fifty-four hours, receiving a total of 325 and 225 mg. ACTH; the eosinophils

returned to pretreatment levels three, four, and six days, respectively, following cessation of therapy. One patient treated at a level of 200 mg. daily for thirteen days developed an elevation in eosinophils to thrice his pretreatment average level coincident with a reduction of his dose to 150 mg.

Pretreatment examinations of the nasal secretions and sputa in three patients revealed high levels of eosinophils; these cells disappeared from the nasal and bronchial secretions between twelve and twenty-four hours after the onset of ACTH therapy.

## 84. ALLERGIC REACTIONS FOLLOWING THE INTRAVENOUS INJECTION OF CORN SUGAR (DEXTROSE OR GLUCOSE)

THERON G. RANDOLPH, M.D., AND JOHN P. ROLLINS, M.D. (BY INVITATION), CHICAGO, ILL., AND CLYDE K. WALTER, M.D. (BY INVITATION), YOUNGSTOWN, OHIO

Pyrogen contamination has been considered as the major cause of reactions associated with intravenous dextrose therapy. Although the institution of sterile vacuum-packed fluid containers and sterile packed disposable recipient sets has largely controlled these factors, disturbing clinical reactions still occur. The fact that these reactions appear in selected individuals suggests that the cause may be due to some peculiarity of the recipient.

So completely have the terms dextrose and glucose been disassociated from their source material that it is not generally realized that *all* dextrose or glucose is prepared from the simple hydrolysis of corn starch. The fact that the ingestion of corn starch and corn sugar (dextrose or glucose) causes allergic responses in corn-sensitive individuals raises the possibility that corn sugar might also act as an allergen when it is injected.

Of several patients, previously shown to be highly corn sensitive, four were selected for this report. In each case the diagnosis of corn sensitivity was made as a result of the experimental feeding of corn meal gruel and corn sugar after four days of complete corn avoidance. Twenty-five cubic centimeters of 5 per cent dextrose were injected slowly intravenously under sterile and pyrogen-controlled conditions. In each case severe constitutional reactions occurred which were clinically similar to those observed following the ingestion of corn sugar and corn meal. The control injection of invert sugar of cane origin failed to produce a similar response with the exception of one patient clinically sensitive to both corn and cane. Similarly, the control injection of normal saline was without effect.

The importance of these observations cannot be dismissed inasmuch as corn sensitivity and wheat sensitivity are of approximately equal occurrence, ranking as the first two foods responsible for the production of chronic food allergy.

Clinical reactions from the intravenous injection of corn sugar occur both in undiagnosed and diagnosed cases of corn sensitivity. More violent reactions may be expected in the corn-sensitive individual who has avoided sources of corn in the diet, either intentionally or inadvertently, for several days prior to the intravenous administration of dextrose or glucose.

## 85. THE DETERMINATION OF THE BASAL METABOLISM BY PERIODIC MAXIMAL EXHALATIONS

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The measurement of the basal metabolism is subject to considerable variation through the natural inability of many subjects to breathe evenly. We have investigated the possibility that the chest position after a voluntarily forced exhalation might be less variable than after a quiet expiration and therefore lead to a more reliable method of estimating the basal oxygen consumption.

One hundred and fifty consecutive subjects of both sexes, with great diversity in age, height, weight, intelligence, and illness, were studied clinically. Their oxygen consumption was measured by the test and standard methods. The test method was a modification of the standard in that every minute the subject was directed to force a maximal exhalation. The first sixty-two subjects were tested by both methods. For the second group of fifty-four subjects a third method was introduced, which modified the test method by continuing for twelve or fourteen minutes and by including early in the test the performance of the maximal breathing capacity. The last group of thirty-four subjects performed the standard and test methods in duplicate. The order in which all tests were done in each series was determined from a prearranged schedule derived from a table of random numbers.

The test procedure does not measurably affect the rate of oxygen consumption. It results in significantly less variation between duplicates. It usually corrects for systematic or irregular deviations in the depth of undirected breathing that commonly lead to either an indeterminate conclusion or to a fallacious inference as to the true rate of oxygen consumption. The uniformity of the end point of forced exhalation is unaffected by such factors as lung disease, congestive heart failure, dysthyroidism, lack of training, or low intelligence of the subject and is less affected by consciousness of breathing than is the standard method.

Therefore this simple modification of the standard method of measuring the basal metabolism allows the rate of oxygen consumption to be estimated in some subjects when the standard method is unreliable, and in all subjects the estimate is usually significantly more precise.

## 86. IDIOPATHIC DILATATION OF THE PULMONARY ARTERY

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Increased interest in the differential diagnosis of congenital heart disease has attracted attention to an unusual disorder characterized by dilatation of the main pulmonary trunk with or without dilatation of the pulmonary arterial branches. This change in the pulmonary artery is unaccompanied by any other structural cardiac or pulmonary disease. It is considered a congenital cardiovascular disorder but its etiology is undetermined. Less than twenty cases which are acceptable by relatively strict criteria have been reported, but there is evidence to suggest that this anomaly is more common than has been assumed.

Five examples of idiopathic dilatation of the pulmonary artery have been observed. In two of them the diagnosis was established at autopsy and in

one of these the diagnosis was made ante mortem. This patient was observed over a period of ten years. The dilated pulmonary arteries were demonstrated angiographically six months before her death. At that time the right, main pulmonary arterial branch was completely occluded by a calcified thrombus. The post-mortem examination disclosed a pulmonary trunk which was actually considerably larger in diameter than the heart itself. The three additional patients are living, but the clinical picture seems compatible with the diagnosis in all respects.

This disorder seems to predominate in female subjects. It has been observed from childhood to old age. Dyspnea, cough, cyanosis, and hemoptysis are common symptoms, although the patient often has few complaints until late in his course, and the final illness may be very brief. The tolerance for exercise and pregnancy is usually surprisingly good. The major abnormalities disclosed by physical examination are a systolic murmur and a systolic pulsation in the pulmonic area, accentuation of the pulmonic second sound, and, often a diastolic murmur along the upper left sternal margin. The abnormally dilated right outflow tract is easily visualized radiographically as well as in angiocardiograms and abnormal pulsations of the pulmonary arteries are common. The electrocardiograms usually show incomplete or complete right bundle branch block or evidence of right ventricular hypertrophy. Recent studies by Cournand and associates have indicated that in these cases the systolic pressure within the dilated pulmonary artery is not elevated and, in fact, is lower than in the right ventricle.

## 87. THE EFFECT OF AUREOMYCIN ON UROBILINOGEN FORMATION AND THE FECAL FLORA

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The effect of aureomycin on urobilinogen formation has been studied in a series of fifteen patients. The amount of fecal urobilinogen was determined serially in seven of these, single determinations being made in the remainder. Bacteriologic studies of the feces were carried out on eleven samples in four cases. In addition, the amount of urobilinogen in the bile obtained by duodenal tube was determined in eight cases.

These studies reveal that, following aureomycin, and concomitant with a great reduction or practical disappearance of coliform organisms in the feces, the urobilinogen of the feces, urine, and bile markedly diminishes, the values often falling into the range which characterizes complete biliary obstruction. The bile urobilinogen was entirely absent in seven of the eight instances, only a trace being demonstrated in the eighth. This may be contrasted with the uniform presence of small amounts in fifty individuals not receiving aureomycin. The Harrison test for bilirubin, applied to the feces, often became positive after aureomycin, but the amounts did not appear to correspond with the reduction in amount of urobilinogen.

In a case of subacute bacterial endocarditis in which urobilinogen studies were first carried out after one month of continuous aureomycin therapy, the feces contained very little bilirubin but gave a fairly intense Ehrlich reaction for urobilinogen. It was possible to demonstrate, however, that no stercobilinogen was present; polarimetry revealed that the only urobilin represented was dextrorotatory rather than levorotatory. It was believed significant that the bile was now Ehrlich negative, indicating that the fecal urobilinogen was not hepatic in origin. The feces at this time contained colon-aerogenes inter-

mediates but no typical *E. coli*. Nonproteolytic clostridia were also present. This combination is of interest since Kämmerer and Miller, and, more recently, Baumgärtel have ascribed the reduction of bilirubin in the colon to *E. coli* and spore-forming anaerobes. In the present study *E. coli* was regularly lacking in the feces after aureomycin and it is evidently essential to the reduction of bilirubin to stercobilinogen. It appears, however, that other organisms of aerogenes type are at times able, perhaps in association with clostridia, to effect a partial reduction of bilirubin, either to mesobilirubinogen or a dextrorotatory derivative thereof.

These results strongly support the belief that the urobilinogens of the feces, bile, and urine are wholly enterogenous. In recent years Baumgärtel and co-workers have described evidence suggesting that, of the two urobilinogens, mesobilirubinogen is hepatogenous, only stercobilinogen being enterogenous. The present study reveals, however, that aureomycin markedly depresses the total urobilinogen formation and that under its influence urobilinogen completely disappears from the bile. These findings are incompatible with the hepatogenous concept unless one were to assume that aureomycin interferes with liver function, for which there is no evidence.

## 88. PAROXYSMAL MYOHEMOGLOBINURIA WITH FATAL RENAL TUBULAR INJURY

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(INTRODUCED BY C. J. WATSON, M.D.)

A fatal case of paroxysmal myohemoglobinuria in a 23-year-old man is presented.

The history was suggestive of a familial occurrence. Fifteen cases have been described in the literature, in three of which there was a history of familial incidence.

Death was due to renal insufficiency and pulmonary edema. The myohemoglobinuria, which had been observed for the first three days of observation, disappeared six days before death. Myohemoglobinemia was carefully looked for but never demonstrated.

In the present case, the myohemoglobin in the urine was identified and distinguished from ordinary hemoglobin by virtue of its spectral distribution curve. Preliminary study of porphyrin metabolism revealed a moderate increase in fecal coproporphyrin, 60 per cent of which was type III.

Significant histologic changes were found in the striated muscles and kidneys. The uremia and subsequent death were the result of tubular injury, as indicated by the demonstration of hydropic degeneration of the proximal convoluted tubules. Although pigment casts were found, they did not appear to be sufficient in number to have been the cause of the uremia, as has been suggested by others. The finding of these casts supports the concept that the precipitation of pigment is more likely the result of disturbed renal function rather than the cause of it.

## 89. THE CLINICAL AND LABORATORY EFFECTS OF HYPOTONIC INTRAVENOUS INFUSIONS

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We have found hypotonic solutions given by vein effective in furnishing water whenever the customary amounts of sodium chloride and dextrose present in standard isotonic solutions were undesirable or unnecessary. The use of hypotonic solutions has, in our experience, been attended with good clinical results and has permitted a more exact maintenance of water and electrolyte balance. Hypotonic solutions of dextrose and/or sodium chloride were first used in correcting disturbances of the extracellular fluid (pattern disturbances, volume losses, and plain-water deficits) in diabetes. Further experience has shown that these solutions are equally valuable in the more seriously ill patients who have a large antecedent plain-water deficit or who have a continuing excessive demand for plain water. For the most part we employed solutions of one-half isotonic strength, and the three most commonly used solutions had the following composition: (1) 25 Gm. of dextrose per liter of distilled water; (2) 4.5 Gm. of sodium chloride per liter; (3) 12.5 Gm. of dextrose and 2.25 Gm. of sodium chloride per liter.

Thus far our clinical experience embraces more than fifty patients who have received hypotonic solutions in amounts varying from 1,000 to 6,000 c.c. in twenty-four hours over periods of from one to twenty-two days.

Immediate observations on the blood were made in twenty-three patients after 1,000 c.c. of a hypotonic solution had been given; twelve patients received 2.5 per cent dextrose and eleven received 0.45 per cent sodium chloride. The infusion was completed in periods of time varying from thirty to ninety minutes. Determinations of the plasma hemoglobin, total plasma protein, plasma specific gravity, hematocrit percentage, the number of erythrocytes, and the hemoglobin were made before the intravenous infusion was started, immediately after it was finished, and one hour after it was finished. As in earlier studies made after the prolonged use of large amounts of these solutions, but on blood drawn four to twelve hours after the last intravenous infusion had been given, none of these immediate determinations showed any evidence of harmful degrees of dilution. A transient, slight fall in their values, shown in most of the determinations made immediately at the end of the intravenous infusion, was followed in the majority by a tendency to rise toward the original levels at the end of one hour. The blood showed no plasma hemoglobin except in two patients; in the one in whom the experiment was repeated with the same intravenous solution, no plasma hemoglobin was found.

## 90. STELLATE BLOCK IN THE MANAGEMENT OF NARCOLEPSY AND CATAPLEXY

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CLEVELAND, OHIO

The effect on narcolepsy of the injection of procaine into the cervical sympathetic chains in four patients with narcolepsy and cataplexy, one patient with narcolepsy, and one patient with hypersomnolence forms the basis of this report. Because of observations on alterations of cerebral function by temporary interruption of sympathetic impulses to the brain, it was decided to ob-

serve the effect in narcolepsy. Associated symptoms and signs in these six subjects have been reviewed, and an attempt has been made to correlate these with the response to stellate blocks. Other common causes for excessive drowsiness were excluded by a number of laboratory procedures. An attempt was made to correlate the response of symptoms to stellate blocks and to the administration of Benzedrine and closely allied drugs.

The urinary excretion of gonadotrophic hormone (F.S.H.) prior to and following stellate blocks was studied whenever possible. An electroencephalogram tracing in one subject discloses a very abnormal deep sleep pattern prior to stellate block. Repetition of the electroencephalogram in this same subject after stellate block injection shows a distinct difference, with a tendency toward a more normal pattern.

In general, patients with narcolepsy and cataplexy have their symptoms controlled for a period of time following stellate block injection, but the effect is more favorable upon the narcolepsy than upon the cataplexy. No change in urinary gonadotrophin excretion was seen to follow the procedure.

## 91. A SYNDROME OF HYPERTENSION, OBESITY, MENSTRUAL IRREGULARITIES, AND EVIDENCE OF ADRENAL CORTICAL HYPERFUNCTION

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Twenty-four women suffering from arterial hypertension were found to possess certain common clinical and laboratory findings which suggested that they belonged to a previously undescribed symptom-complex. These were: (1) relatively sudden onset of obesity occurring at menarche, menopause, after multiple pregnancies or gynecologic operation; (2) obesity of the "central type," with pale striae on thighs and sometimes on upper arms; (3) menstrual irregularities; (4) good or excellent therapeutic response of blood pressure levels to diets low in salt, with reversals when the intake of salt was raised; (5) abnormally low concentrations of sodium and chloride in sweat. Secondary and less common signs and symptoms included an aversion to salty foods and high fluid intakes, unexplained periods of oliguria, inconstant glycosuria or a diabetic type of glucose tolerance curve which was accompanied usually by increased sensitivity of the blood sugar levels to intravenously injected insulin, plasma chloride levels above or at upper limits of normal, hirsutism, a tendency to easy bruising and ecchymoses, a relatively benign type of hypertension with little albuminuria or other signs of renal damage, and sensitivity of the blood pressure to injected desoxycorticosterone acetate or glucoside. In no instance were the cardinal manifestations of Cushing's syndrome present. Because these symptoms, signs, and findings were seen rarely in other types of hypertension and because concentrations of sodium chloride in sweat were normal in most subjects, including obese nonhypertensive women, it is believed that these patients represent a separate pathogenetic entity. The evidence is strongly suggestive that certain functions of the adrenal cortex are specifically disturbed in these patients, but not in other forms of hypertension or obesity.

## 92. A LONG-TERM EVALUATION OF THE THERAPY OF PERNICIOUS ANEMIA WITH FOLIC ACID

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Folic acid (pteroylglutamic acid) was introduced in 1945 as a specific therapeutic agent for "liver extract principle" deficiency diseases. Numerous short-term observations quickly confirmed its value. Because the natural history of pernicious anemia is characterized by exacerbations and remissions, it was felt that long-term studies were necessary in order to critically evaluate any new therapeutic agent.

Early in 1946, ninety-eight patients with pernicious anemia were selected for such a study from the Out-patient Anemia Clinic of the Cook County Hospital. Their course, liver extract requirements, neurological status, and other characteristics were well established, the patients having been followed by the same group of observers and maintained in remission with liver extract for periods ranging from months to years. Folic acid was administered orally, in a daily dose of 5 milligrams. The patients were seen at four-week intervals for interviews and neurological and hematologic examinations.

The present report summarizes the observation made during the three and a half years the study has been in progress. There have been thirty-one neurological and thirty hematologic relapses, with eight patients showing a combination of both. In three patients the folic acid was discontinued because of persistent weight loss and/or soreness of the mouth. Twenty-nine patients discontinued their therapy for reasons beyond our control. Most of these patients disappeared from observation, many, however, only after insisting that they felt better while on parenteral liver therapy which they wished resumed. Of the ninety-eight patients only thirteen have been able to remain on continuous oral folic acid therapy and maintain themselves in remission.

Without exception, every hematologic relapse was quickly reversed with a change to parenteral liver therapy. In fourteen patients with neurological relapse, however, the changes have not been reversible in spite of intensive therapy with liver extract for six months or more. Bone marrow aspirations were performed in five patients at the time of relapse. Megaloblastic changes were demonstrable in all instances.

The original plan envisioned an attempt to increase the dose of folic acid in those patients who showed evidences of relapse, but the impression (gained from the current reports of the time) that larger doses of folic acid precipitated neurological relapses faster than small doses deterred us.

## 93. HEMOPOIETIC CHANGES DURING CHLOROMYCETIN ADMINISTRATION

ITALO F. VOLINI, M.D., STEVEN O. SCHWARTZ, M.D., IRVING GREENSPAN, M.D. (BY INVITATION), LEE EHRLICH, M.D. (BY INVITATION), JAMES A. GONNER, M.D. (BY INVITATION), AND OSCAR FELSENFELD, M.D. (BY INVITATION)
CHICAGO, ILL.

Toxic manifestations of oral administration of chloromycetin have not been reported. Recently we have observed three patients in whom hematologic abnormalities were encountered during chloromycetin therapy.

Two patients with typhoid fever and one with acute brucellosis were treated with oral chloromycetin for periods ranging from nine to nineteen days. The total dose of chloromycetin ranged from 30 to 60 Gm. and was given in a uniform dose of 3 Gm. per day (0.5 Gm. every four hours).

A significant fall in the white blood cell count, attributable to the diminution in the number of granulocytes, could be demonstrated as early as the sixth day of therapy. White cell counts as low as 3,000 and absolute neutrophil counts of 264 were observed. No significant changes in the total number of lymphocytes were demonstrated. A slight drop was seen in the red cell count and the hemoglobin. In one instance this drop was out of proportion to a fall ascribable to the underlying infection.

The marrow was studied in all patients. In the first, a marked granulopenia and left shift in erythropoiesis were present on the eleventh day of therapy. In the second patient the marrow was studied three and thirteen days after the discontinuance of the chloromycetin. On the third day a left shift in granulopoiesis as well as erythropoiesis was seen, with an increase in eosinophils. By the thirteenth day the left shift had disappeared in both series and evidences of marked regeneration accompanied by an extraordinary eosinophilia were seen. In the third patient, the marrow was studied at the beginning of therapy and revealed the changes expected with infection. Eleven days later (two days after discontinuance of therapy) granulopoiesis had markedly diminished and a left shift had occurred in erythropoiesis.

Upon the discontinuance of the chloromycetin there was an immediate spontaneous reversal of the downward trend in the blood values.

In the three patients studied there is evidence that within a few days chloromycetin produces a marrow hypoplasia more marked in the granulocyte series but also involving a maturation arrest of erythroid elements. The peripheral blood simply reflects the more fundamental changes in the marrow. In the therapeutic doses employed in the present series the marrow alterations are reversible.

Both clinical and experimental studies are in progress to verify and extend these findings.

## 94. EXPERIMENTAL SYPHILIS IN THE RABBIT: THE RELATIONSHIP OF METACHROMASIA TO FIBRINOID DEGENERATION OF COLLAGEN AND THE LOCALIZATION OF SPIROCHETES IN THE TESTIS

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ST. LOUIS, MO.

Since the earliest studies of experimental syphilitic orchitis there has been recognized, as a characteristic feature of the inflammatory reaction in the testicular stroma, the deposition of a relatively acellular, loosely constructed material resembling Wharton's jelly. The term "mucous (or mucoid) degeneration" of the connective tissue has been applied to this change. The material stains metachromatically and has been found in greatest abundance in the tunica albuginea, particularly around blood vessels.

We have confirmed the preceding observations in a study of syphilitic orchitis in the rabbit using the Nichols' strain of *T. pallidum*. In addition, we have found that previous incubation of the sectioned tissue in hyaluronidase (Schering) abolishes the capacity for metachromatic staining with thionin and with toluidine blue. No metachromasia is found in the stroma of the nor-

mal testis. Focal fibrinoid degeneration of collagen has been observed in the metachromatic areas and the highest concentration of spirochetes has been noted in and around these sites.

It may be concluded that the loosely constructed, relatively acellular material which appears in large amounts in the stroma of the syphilitic rabbit testis and which stains metachromatically contains hyaluronic acid or a closely related substance. It would appear that the sites of localization of the metachromatically staining material, the fibrinoid degeneration of collagen, and the highest concentrations of spirochetes are intimately related spatially. A sequential study of syphilitic orchitis in the rabbit is now in progress to investigate the possible causal or temporal relationships between the multiplication of spirochetes, the development of metachromasia, and the appearance of fibrinoid degeneration of collagen.

## 95. CONTINUOUS DICUMAROL PROPHYLAXIS IN CORONARY DISEASE

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It seemed reasonable to suppose that an agent which would prolong coagulation time and have a deterrent effect on blood sludging might be of value in preventing thrombosis in chronic arterial disease. Accordingly, twenty-one patients have been given Dicumarol continuously for periods of three weeks to three years. Of these, eighteen had clinical and electrocardiographic evidence of coronary thrombosis with myocardial infarction, two had coronary sclerosis with angina of effort, and one had cerebral thrombosis with left hemiparesis.

After initial stabilization, prothrombin times were determined weekly by Quick's method, and Dicumarol was prescribed in daily doses sufficient to maintain prothrombin time between two and two and a half times normal control values. Individual requirement varied from 50 mg. every other day to 150 mg. daily.

Five patients showing marked sensitivity to Dicumarol and unable to take as much as 50 mg. daily without alarming fluctuation in prothrombin time were given menadione bisulfite, 2 mg. daily. These showed increase in tolerance, and prothrombin times thereafter were more readily controlled.

All patients were advised of the risks involved and instructed in the symptoms and signs of overdosage.

No disastrous toxic effects have been encountered. Transient hematuria occurred in three patients; rectal bleeding from hemorrhoids which had bled prior to therapy occurred in one patient; cutaneous ecchymoses occurred in three patients; epistaxis occurred in one patient. One man with previous history of bleeding duodenal ulcer bled following extraordinary dietary indiscretion although his prothrombin time was within the desired range. The drug was discontinued and coronary thrombosis recurred within ten days. Dicumarol was then resumed and he has been well and at work for the past ten months.

There have been no deaths in the series. Chest pain recurred in two patients when the drug was discontinued because of toxic effect and ceased when Dicumarol was resumed. Anginal attacks have ceased entirely in eight patients and have been markedly reduced in frequency and severity in two. All of the patients have resumed their former activities.

Electrocardiograms in five cases have shown a striking reversion toward normal.

It is surprising and worthy of note that all patients have followed instructions carefully and have expressed the desire to continue under treatment indefinitely in spite of the inconvenience involved.

Results seem to indicate that continuous and controlled Dicumarol therapy is practicable and may influence favorably the course of disease of the coronary arteries.

## 96. THE USE OF ORAL MERCUHYDRIN COMBINED WITH ASCORBIC ACID IN CARDIAC DECOMPENSATION

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The effect of the oral administration of a tablet containing 60 mg. of an inorganic mercurial compound (Mercuhydrin), equivalent to 19.5 mg. of mercury, and combined with 100 mg. of ascorbic acid has been studied in one hundred patients with congestive heart failure. The patients' ages ranged from 12 to 60 years and the congestive failure was due to valvular, hypertensive, or arteriosclerotic heart disease. From one to three tablets were administered daily.

These tablets were equally effective as one containing 120 mg. of Mercuhydrin alone. They were satisfactory in controlling mild congestive failure in approximately one-third of the patients when used as a primary treatment. They were effective in controlling mild to moderate congestive failure in approximately two-thirds of the patients after maximum compensation had been obtained following injectable Mercuhydrin. They were usually ineffective in controlling severe failure when used alone, but frequently of value when used as a supplement to injections. Toxicity, when it occurred, was mild and transient in the large majority of instances.

The studies indicated that the tablets may be used either alone or as a supplement to parenteral therapy to assist in the control of congestive heart failure.

## 97. SMALL BOWEL CHANGES IN AMEBIASIS

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ERNA LEWIN-ARENDT, M.D. (BY INVITATION), CHICAGO, ILL.

Following an attack of amebiasis, many patients manifest a multiplicity of abdominal symptoms which, though nondescript and not too severe, frequently make them gastrointestinal problems. The frequent finding on cytologic examination of the stool of epithelial cells originating from the small bowel suggested the latter as the possible site of "lesions" leading to the mentioned symptoms. Hence, all patients with chronic amebiasis had x-ray films made of both the small and the large bowel for possible pathologic changes.

Of sixty patients with chronic amebiasis thus examined, twenty-seven showed changes from the normal in the small bowel, particularly the terminal ileum. These changes are not seen as a rule in ulcerative colitis, sprue, and various nutritional deficiencies.

Spasm and edema of the terminal ileum are the changes most often noted. More characteristic, however, are the edematous folds of the terminal ileum which sometimes made the terminal ileum appear almost as wide as the colon.

The nonspecific changes of segmentation and pooling as seen in sprue or the ulcerated involvement of the terminal ileum as seen in ulcerative colitis were not observed in these patients. The motility of the small bowel appeared undisturbed.

The patients demonstrating the described changes in the terminal ileum were in general more difficult to manage clinically, especially with regard to control of the cramping abdominal pain.

## 98. PHYSIOLOGIC AND PHARMACOLOGIC STUDIES IN A CASE OF PHEOCHROMOCYTOMA

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A case of pheochromocytoma, subsequently proved at operation and at autopsy, allowed us the opportunity to compare in a single patient the physiologic and pharmacologic specificity of a number of suggested diagnostic procedures. The patient, a woman with persistent hypertension for approximately one year, preceded by at least three years of paroxysmal hypertension, had a blood pressure which ranged from 215/140 to 250/155 mm. Hg.

Benzodioxane (933F) produced an immediate fall in pressure to normotensive levels. Dibenamine produced a similar fall, which was slower in onset and persisted longer. Tetraethylammonium chloride (TEAC) produced a sharp rise in pressure with failure to return to initial levels for some time; this rise was shown to be abolished by benzodioxane. Diminished sensitivity to the pressor activity of intravenous adrenalin was demonstrated. Histamine and mecholyl each caused a fall in pressure, the rise reported to be characteristic of pheochromocytoma being absent.

The results bear out the specificity of benzodioxane and Dibenamine in counteracting the pressor activity of circulating epinephrine. Response to benzodioxane seems more specific, however, because no depressor effects have been encountered in a series of control hypertensive subjects, whereas such reactions do occur with Dibenamine. Diminished sensitivity to exogenous adrenalin would also appear to have specific meaning in that tolerance to the effect of this drug appears to develop when it is present in the circulation in large amounts. The pressor response to TEAC would appear humoral in nature, specifically adrenergic in this instance since it was abolished by benzodioxane. The lack of a pressor response in this case to either histamine or mecholyl suggests that these tests are not specific for diagnosis.

The results suggest the specificity of benzodioxane in the diagnosis of pheochromocytoma, when the pressure is elevated, and the desirability of evaluating the mechanisms of pressor responses to histamine, TEAC, or other stimuli by administration of benzodioxane.

## 99. HEART BLOCK FOLLOWING MEDULLARY PERFUSION WITH BACTERIAL TOXINS

JOHN A. SHEEDY, M.D. (BY INVITATION), AND N. C. GILBERT, M.D.
CHICAGO, ILL.

The role of bacterial and viral agents in the production of heart block has been known for many years. Of particular interest has been the occurrence of bradycardia and heart block following acute bacterial infections, es-

pecially in the case of diphtheria. The question of whether myocardial damage per se or increased vagal tone or a combination of both is responsible has remained to conjecture, with the preponderance of opinion in support of cardiac damage. No adequately controlled experimental evidence has thus far been presented to note the effect of increased vagal tone in the pathogenesis of heart block.

Six commercially produced bacterial toxins were employed in this study, namely: *C. diphtheriae, Staph. aureus, Str. hemolyticus, Cl. tetani, Cl. welchii,* and *Cl. botulinum.* These toxins were perfused through the turtle brain which was connected with the body of the animal only by means of the vagus nerves.

Heartblock was produced with all of the toxins. The blocks produced by *C. diphtheriae, Cl. welchii,* and *Cl. botulinum* could be released by atropine. These results suggest an acetylcholine-like mechanism. The remaining toxins were not affected by atropine and the mechanism for their action is unknown.

## 100. THE TAKATA-ARA REACTION IN THE DIFFERENTIAL DIAGNOSIS OF JAUNDICE

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In the work-up of jaundiced patients the Takata-Ara reaction (TAR) seemed of diagnostic help in certain types of jaundice; hence, an attempt was made to re-evaluate the differential diagnostic significance of the TAR on the basis of recent classification of hepatobiliary diseases.

The TAR was performed in 356 cases and compared with the results of cephalin-cholesterol flocculation, thymol turbidity, and zinc sulfate turbidity tests. The percentage of positive TAR's in the different types of hepatobiliary disease were as follows: Laënnec's nonfatty cirrhosis, 87.8; fatty cirrhosis, 35.5; postnecrotic (nonalcoholic) cirrhosis, 100; infectious viral hepatitis, 10.7; homologous serum hepatitis (HSH), 68.0; toxic hepatitis, 27.6; obstructive jaundice (incomplete, benign), 2.0; and obstructive jaundice (complete, malignant), 20.0.

The TAR may therefore assist not only in differentiating a cirrhosis with jaundice from an acute hepatitis but also from an infectious hepatitis. The TAR is not related to the degree of icterus, and the high incidence of abnormal TAR's in HSH is therefore not an expression of the well-known greater severity of the latter.

Moreover, no parallelism was found between the TAR and the cephalin-flocculation and thymol turbidity tests. A moderate parallelism existed between the TAR and the zinc sulfate turbidity, except in infectious hepatitis where the latter is moderately elevated and the TAR is negative; in toxic hepatitis this exception is less marked.

A discrepancy between the TAR and the zinc sulfate turbidity is also apparent during the recovery phase of hepatitis when the zinc sulfate turbidity increases while the TAR (if positive) becomes negative. While, in recovering hepatitis, a rise in the zinc sulfate turbidity and a fall in the cephalin-flocculation and thymol turbidity occurs, a rise in the TAR in addition (or the maintenance of a positive TAR) speaks for the transition from hepatitis to cirrhosis. Similarly, the appearance of positive TAR in a fatty cirrhosis seems to indicate progression to a Laënnec's nonfatty cirrhosis.

The TAR may indicate certain phenomena not demonstrable by the other flocculation tests.

## 101. A STUDY OF THE MOVEMENTS AND SOUNDS OF HEART VALVES OF VARIOUS LABORATORY ANIMALS (A MOTION PICTURE AND SOUND RECORDING)

HARRY L. SMITH, M.D., HIRAM E. ESSEX, PH.D. (BY INVITATION), AND EDWARD J. BALDES, PH.D. (BY INVITATION), ROCHESTER, MINN.

The hearts of various laboratory animals were perfused with oxygenated Ringer-Locke solution and were kept beating for various lengths of time. Rather large openings were made in the walls of the auricles. This afforded an excellent view of the entire valves. Colored motion pictures were made of the exact movements of the mitral, tricuspid, aortic, and pulmonic valves. A sound record, an electric record of the sound, and an electrocardiogram were made at the same time the motion picture was made. These records enable one to see the movements of the heart valves, to hear the sounds they produce, to see an electric record of the sounds, and to see an electrocardiogram recorded at the same time. The results of this study furnish information that we believe will change some of our previous ideas about the factors that cause the mitral and tricuspid valves to open and close.

## 102. CARDIODYNAMIC AND RENAL CHANGES IN SPONTANEOUS AND NEPHROGENIC HYPERTENSIVE DOGS IN RESPONSE TO TISSUE INJURY

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CHICAGO, ILL.

Tissue injury, particularly abscess, produces a sustained fall in blood pressure in some spontaneous and nephrogenic hypertensive dogs. This study was undertaken to investigate the cardiodynamic and renal alterations ensuing during this depressor response.

The following determinations were done before and after various types of tissue injury: (1) renal plasma flow (R.P.F.), (2) glomerular filtration rate (G.F.R.), (3) plasma volume, (4) thiocyanate space, (5) central venous, right atrial, right ventricular, and pulmonary artery pressures, (6) blood pressure.

It is concluded from the data that: (1) Spontaneous and nephrogenic hypertensive dogs exhibit the same hemodynamic pattern. The cardiac output, plasma volume, thiocyanate space, and pulmonary artery pressure are all within normal ranges. Both types of hypertension are due to an increase in peripheral resistance. (2) Spontaneous hypertensive dogs all have normal G.F.R. and R.P.F. In contrast, some nephrogenic hypertensive dogs have normal clearances; others exhibit significantly reduced kidney function. (3) A sustained blood pressure fall can be readily elicited by injection of abscess-inducing substances such as turpentine, indicating that the depressor response is a nonspecific one with respect to presumed renal hypertensive mechanisms. (4) Accompanying the sustained depressor response in both nephrogenic and spontaneous hypertensive dogs is a sustained increase in R.P.F. G.F.R. does not change significantly. Renal vascular resistance, particularly efferent arteriolar, is reduced. (5) The plasma volume, thiocyanate space, venous pressure, and right heart pressures are not affected by tissue injury. The blood pressure fall is not due to hypovolemia. (6) The cardiac

output is increased during the depressor response to injury. The fall in blood pressure is therefore due to a decreased peripheral resistance. Apparently the decrease in both renal and general peripheral resistance is the resultant of a common train of events elicited by injury. There is no evidence for a cause and effect relationship between them. The blood pressure fall following injury cannot be attributed to "relief of renal ischemia."

## 103. CLINICAL EVALUATION OF A NEW LIPASE PREPARATION

M. H. Streicher, M.D., Verna Pittard (By Invitation), and Betty Woodson (By Invitation), Chicago, Ill.

Many agents are available which have the property of digesting fat, yet there is a need for one which would be capable of hydrolyzing a wider variety of lipids.

It was thought, on the basis of experimental evidence in animals, that lipase A may be helpful in individuals who display an inability to adequately digest all forms of food fat. The new preparation is an enzyme, protein in nature, produced during the growth of a certain type of mold (fungus).

In this study lipase A was used in patients having steatorrhea. Control studies also were made. Each capsule contained 0.4 Gm. of the enzyme and each patient was given twenty-four capsules daily.

These individuals were placed on a calculated diet of low fat (50 Gm. in twenty-four hours) and evaluated clinically and chemically before and after lipase was given. The chemical determinations were made on the nitrogen, on the total and neutral fat and fatty acids of the stool; the blood was studied as to content of lipase, calcium, nonprotein nitrogen, and total protein, and the urine was studied as to total output of nitrogen. These determinations were made before and after the fat content was changed in the diet.

Comparative study was also made with the use of pancreatin in the same group of patients.

A specific example will demonstrate the efficacy of the new enzyme.

A patient with carcinoma of the pancreas was placed on a 55 Gm. fat diet plus adequate protein and carbohydrate. The total fat calculated in the stool before lipase A was administered was 57.0 per cent (dry method). After three capsules of lipase A were given per day for a period of three weeks, the total fat in the stool was reduced to 42.0 per cent. After the diet was increased to 152 Gm. fat, the stool fat was elevated to 73.0 per cent. The administration of twelve to twenty-four capsules of lipase decreased the total fat in the stool to 53 per cent and 49 per cent respectively.

Clinically, the patient improved, the stools decreased in number and became more solid, and the cramping in the abdomen subsided. In general, the patient ate better and felt better.

*Conclusion:* (1) Lipase A is helpful in steatorrhea. (2) Large quantities of the enzyme are required for optimum results. (3) Pancreatin compares favorably with results obtained with lipase A.

## 104. ANGIOCARDIOGRAPHY

GEORGE C. SUTTON, M.D., GEORGE WENDELL, M.D., HARRY GRANT, M.D., AND HAROLD WEDELL, M.D., CHICAGO, ILL.

(INTRODUCED BY DON C. SUTTON, M.D.)

A comparison is made between angiocardiography by arm vein and direct catheterization of the heart.

Direct intracardiac angiocardiography is a procedure possessing few difficulties and of negligible risk. The procedure will give contrast visualization of the heart and great vessels which is routinely superior to that obtained by peripheral injection of a radio-opaque material. This is especially true for levoangiocardiograms. Simple, inexpensive equipment for changing the films and low power x-ray apparatus are more practical for use with intracardiac injection, without loss of a very high percentage of diagnostic films. In some cases demanding a high concentration of contrast media and accurate timing of exposures, only intracardiac injection will produce satisfactory angiocardiograms.

## 105. DIVISION OF THE POPLITEAL VEIN IN VALVULAR INSUFFICIENCY OF THE DEEP VENOUS SYSTEM OF THE LOWER EXTREMITIES

GEZA DE TAKATS, M.D., AND GUSTAV W. GRAUPNER, M.D. (BY INVITATION)
CHICAGO, ILL.

From a large number of patients exhibiting valvular incompetence of the deep veins, six patients have been selected to test the possibility that the division of the popliteal vein overcomes the "bursting" type of pain on standing and decreases or eliminates postural edema. All patients have had a deep thrombophlebitis with recanalization, subsequent edema, induration, and ulceration. All have been under our care for several years and were treated by compression bandages, excision of ulcers, skin grafts, or saphenous vein ligation, in spite of which there were recurrences. Since previous experience with femoral vein ligations gave no appreciable help, division at this site was done with the following results: (1) all six patients lost their "bursting" pain on standing, (2) swelling was greatly decreased or completely abolished in every instance, (3) patches of recurrent lymphedema with ulceration were uninfluenced, this complication requiring other forms of therapy. This simple procedure may eliminate the incapacitating sequelae of postphlebitic edema in selected cases.

## 106. QUANTITATIVE STUDIES OF TREATMENT OF ACUTE CLOSED CEREBRAL INJURY BY HYPERTONIC INTRAVENOUS GLUCOSE OR SURGICAL DECOMPRESSION

C. BRUCE TAYLOR, M.D., AND GEORGE M. HASS, M.D., CHICAGO, ILL.

Acute, closed, intracerebral lesions characterized by hemorrhage, necrosis, and progressive edema were produced in rabbits by freezing the brain through the intact skull with an instrument cooled by expanding carbon dioxide. The dimensions and locations of cerebral lesions were controlled so that they could be reproduced in successive animals.

Volumes of cerebral damage, ranging from 1.3 to 32.5 vol. per cent of the brain, were produced in one hundred four untreated animals. In this group, the minimum lethal quantity of damage was 9.4 vol. per cent. No animal survived with damage in excess of 18.0 per cent. The average minimum lethal quantity of damage was 14.3 vol. per cent. Most animals that died had a normal postoperative period of behavior. Secondary lapse into stupor was a dependable indication of eventual death within two hours.

Twenty-five animals with acute, closed, cerebral lesions were treated with intravenous injections of hypertonic aqueous solutions of glucose given continuously or by single or multiple infusions. Intravenous infusions were started shortly after the production of lesions. This therapy was of no benefit, either from the point of view of amelioration of symptoms or survival.

When surgical decompression was done with removal of bone-flaps over cerebral lesions within forty-five minutes after production (forty-seven animals) or after onset of stupor, coma, or convulsions (eight animals), symptoms were relieved and animals survived with more cerebral damage than control, untreated animals. In these animals the minimum lethal quantity of cerebral damage was 14.6 vol. per cent. The maximum survivable quantity of cerebral damage was 32.7 vol. per cent. The average minimum lethal quantity of cerebral damage was 21.9 vol. per cent which was about 50 per cent greater than that in the untreated group (14.3 vol. per cent) and in the group treated with hypertonic solutions of glucose (14.5 vol. per cent). Four animals treated by contralateral surgical decompression had no increased tolerance to cerebral damage.

These data indicate that hypertonic solutions of glucose, given periodically or continuously by the intravenous route, were of no value in the treatment of acute, closed, cerebral lesions characterized by local hemorrhage, necrosis, and edema. Surgical decompression was of great benefit when done over the site of the lesion. It was concluded that surgical decompression of the type used should be evaluated as a method of treatment of patients with localized intracerebral necrosis, hemorrhage, and edema due especially to vascular disease.

## 107. FURTHER EXPERIENCE WITH BACTERIAL PYROGENS IN THE TREATMENT OF MALIGNANT HYPERTENSION

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Of eleven patients with malignant hypertension showing reversal, eight are alive without further treatment and lead useful lives. The average survival time for this group is now thirty-two months. Two patients have died of cerebral hemorrhage thirty-seven and sixteen months after treatment, and one has not been re-examined.

Six patients have been treated during the past year. Of these, three had $Tm_{PAH}$* values which exceeded 40 mg. per minute. The blood pressure of the first patient was reduced from 175/118 to 140/84 mm. Hg during six weeks of treatment. All signs of malignant retinopathy receded; the electrocardiogram and teleroentgenogram became normal; and $Tm_{PAH}$ increased from 55 to 77 mg. per minute. There have been no further signs of malignancy.

The next two patients did equally well for seven and eight weeks of treatment. Papilledema receded from Grade III to Grade I; hemorrhages and exudates cleared completely in one and decreased from Grade II to Grade I severity in the other.

*Tubular mass measured by para-aminohippurate excretion.

These two patients objected to prolonged hospitalization, hence elected to discontinue treatment. One died during the second stage of lumbodorsal sympathectomy; the other shows no signs of progression of the disease.

A fourth patient had a $Tm_{PAH}$ of only 33 mg. per minute. He had Grade IV retinopathy, gross hematuria, and electrocardiographic and x-ray signs of hypertensive heart disease. He was treated for two months. The blood pressure was reduced from 204/125 to an average of 140/90. All signs of retinopathy and cardiac disease disappeared. Maximum specific gravity increased from 1.016 to 1.028.

Two patients having Tm (tubular mass) values of 25 and 9 mg. per minute have done poorly. One died of cerebral hemorrhage during treatment and the other is showing signs of progressive renal failure, although the eye grounds and blood pressure now are improved.

Renal function with $Tm_{PAH}$ values of at least 33 mg. per minute continues to be the best pretreatment index of satisfactory response.

The pyrogen Pyromen is given by vein in doses large enough to cause a daily temperature rise to 102 to 104° F. The initial dose is 0.5 c.c. of solution containing 50 gamma of solids per cubic centimeter. Thereafter, the amount given is determined by the temperature peak of the preceding day. The daily injections should be continued as long as the blood pressure falls and signs of malignant hypertension regress.

When the blood pressure is no longer decreasing, the frequency of injections may be reduced. Over a period of two to six months there is usually a gradual rise in pressure in most of the patients and eventually hypertensive levels may prevail. However, signs of malignancy have so far not returned and the patients can be dealt with as having uncomplicated essential hypertension.

## 108. PNEUMOCOCCUS TYPES, MORTALITY, BACTEREMIA, AND PURULENT COMPLICATIONS IN PRIMARY PNEUMOCOCCIC PNEUMONIA AT THE CINCINNATI GENERAL HOSPITAL, 1936-1949

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Three thousand one hundred four cases of primary pneumococcic pneumonia in patients over 13 years of age with specific typed pneumococci in sputum or blood cultures are reported here. Pneumococcus typing has been carried out as a means of specific diagnosis to evaluate various modes of therapy. The data for each year are calculated from July 1 through June 30 the following year.

The number of cases per year decreased by gradual fluctuation from three hundred eighty-seven in 1936-37 to one hundred twenty-two in 1948-49.

Type 1 was most frequent in 1936-37, 1938-39, 1939-40, 1940-41, 1942-43, 1943-44, and 1948-49. Types 1 and 7 were equally frequent in 1944-45. Type 7 was most frequent in 1941-42, 1945-46, and 1946-47. Type 3 was most frequent in 1937-38, and Type 2 was most frequent in 1947-48.

Mortality was 36.7 per cent, 32.4 per cent, and 28.1 per cent respectively for 1936-37, 1937-38, and 1938-39 when antipneumococcic serum for certain types was the only specific treatment. However, sulfapyridine came into use in January, 1939. From July 1, 1939, through June 30, 1947, sulfonamides were the mainstay of treatment. For these eight years mortality ranged from 15.3 per cent to 12.6 per cent, except that mortality was 20.2 per cent in 1940-41,

19.5 per cent in 1943-44, and 18.6 in 1944-45. In 1945-46 and 1946-47 penicillin was used for treatment if there was no response to sulfonamide, or if sulfonamide drugs were contraindicated. Penicillin was used exclusively for treatment in 1947-48 and 1948-49. Mortality was 9.7 per cent in 1947-48, and in 1948-49, when aqueous crystalline penicillin in doses of 200,000 or 300,000 units was given intramuscularly twice on the first day of treatment and once daily thereafter, mortality was 5.7 per cent.

Pneumococcus bacteremia was found in 12.9 per cent of the cases in 1940-41, and in 49.5 per cent of the cases in 1947-48. In the remaining years bacteremia was found in 16.4 to 31.8 per cent of the cases.

Empyema, meningitis, endocarditis, lung abscess, or pyarthrosis occurred in 13.2 per cent of the cases in 1937-38, and in 3.8 per cent of the cases in 1940-41. In the remaining years these complications occurred in 4.6 to 10.7 per cent of the cases.

Apparently penicillin therapy has reduced mortality, and the unchanged frequency of bacteremia and purulent complications results from the onset of these manifestations before the start of treatment.

## 109. CONTROL COMPARISON OF NU-2206 (3-HYDROXY-N-METHYLMORPHINAN HYDROBROMIDE) WITH MORPHINE SULFATE FOR RELIEF OF POSTOPERATIVE PAIN

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In an earlier investigation of the analgesic properties of NU-2206 (3-hydroxy-N-methylmorphinan hydrobromide) the effect of this agent on the pain threshold in normal volunteer student subjects was determined. There followed a clinical trial to determine its effect in a variety of patient categories. It was found to have desirable analgesic properties with an apparently more prolonged effect than that of morphine sulfate in equivalent dosage ranges. It was found that the dosage range was from 3 to 6 mg. as compared with 10 to 15 mg. of morphine. The side effects did not appear to be severe or frequent. A limited study was made on a small number of patients who had received the drug over a prolonged period of time, and no withdrawal symptoms were observed.

Its prolonged analgesic effect appeared beneficial in those patients who required protracted relief and in whom frequent injections with shorter-acting analgesics were undesirable. Controlled study then was undertaken on ward patients undergoing surgical procedures on the General Surgical and Urological services. Those under the age of five years or in such condition as to be outside the preselected dosage range were excluded. Patients having intracranial surgical procedures also were omitted. They were divided into fifteen operation categories so that subsequent statistical evaluation as to the amount of pain associated with various types of operative procedures could be compared. In each operation category alternate patients were given NU-2206 or morphine sulfate. The medications were so dispensed as to preclude identification except by code letter.

The nursing staff was instructed to give 1 c.c. for each patient or ½ c.c. for each patient. One cubic centimeter contained either 5 mg. of NU-2206 or 10 mg. of morphine sulfate. The drugs were administered by the nursing staff to be given as required for the relief of pain not more frequently than every three hours. A special form was provided listing seven common complications

ordinarily seen in conjunction with the use of analgesic drugs. The nurses' observations were checked closely by the same member of the staff each day.

It appears, after studying 406 patients by this method, that analgesic drugs are not needed in slightly more than one-third of the instances. A difference in duration of action of NU-2206 and morphine sulfate was not demonstrated. NU-2206 is as effective an analgesic agent as morphine, with no demonstrable increase in side actions.

## 110. SPONTANEOUS RUPTURE OF THE HEART FOLLOWING ACUTE MYOCARDIAL INFARCTION

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Cardiac rupture occurred in eight patients (7.2 per cent) of a series of 111 instances of acute myocardial infarction in patients who had been observed in the Evanston Hospital and came to autopsy during the past six years.

The eight patients were all of the white race. Their ages ranged from 51 to 86 years, averaging 68, and they were equally divided in sex. Half of the group denied prior angina, three had experienced pains from one to four months, and one who died within two days after onset of illness gave no history of anginal pain or hypertension and had considered herself in excellent health. However, there was evidence of old infarction in four of these patients.

Hypertension persisted after the onset of clinical myocardial infarction in all but one of these patients, and he belonged in the group of four who gave no prior history of hypertensive disease.

Death occurred rapidly after the occurrence of the rupture as evidenced by the condition of the pericardial sac and lacerated tissues. Four patients showed ante-mortem clotting of the blood in the pericardial sac and one had organization of the clot, but the remaining three revealed only bright, unclotted blood.

Clinical observation indicated that three expired immediately after the rupture, four within a day, and only one survived sixty hours. All eight deaths occurred within two to eleven days, average 5.8, after the clinical onset of acute myocardial infarction. The rupture occurred in the left ventricle in seven of the patients, four posteriorly and three anteriorly, while the remaining one ruptured through the right ventricle and septum at the apex.

Positive electrocardiographic evidence of acute myocardial infarction was present in all of the rupture group except one patient on whom such an examination was not possible because she expired within a half hour after admission to the hospital. The clinical diagnosis of rupture was made in six of the patients prior to autopsy.

Rupture of the heart occurred in four of these patients during defecation.

The leucocyte counts and blood sedimentation rates were consistently above normal levels in both the rupture and nonrupture groups, but there was no significant difference.

## 111. CLINICAL AND LABORATORY OBSERVATIONS IN FATTY INFILTRATION OF THE LIVER

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Fifteen patients with pure fatty infiltration of the liver demonstrated by needle biopsy were selected for study.

Thirteen of the fifteen patients were admitted to the medical service for various causes among which were hematemesis, pellagra, chronic cholangitis, diabetes mellitus, chronic pancreatitis, xanthomatosis, congestive heart failure, myocardial infarction, malnutrition, and gastritis. Two patients with mycosis fungoides and discoid lypus erythematosis respectively were studied on the dermatologic service.

The ages of the patients ranged from 29 to 85 years. Nine of the patients were women. A definite history of alcoholism was present in six. The diet was adequate in only six patients. An antecedent history of jaundice was obtained in two patients, both of whom had had previous cholecystectomies. Mild diabetes mellitus was present in two, while obesity was present in six patients. A history of weight loss of 10 pounds or more was obtained in ten of the subjects. Nausea and vomiting occurred in six, while anorexia was prominent in ten.

Hepatomegaly (ranging from one to five fingerbreadths) was observed in ten of the fifteen patients, while splenomegaly was present in only one instance. Edema of the lower extremities was encountered three times. Glossitis and evidences of peripheral neuritis were found in two patients.

Laboratory studies showed an elevation of the thymol turbidity in five of fourteen cases and a 3 to 4 plus cephalin cholesterol flocculation test in seven of fourteen cases. Serum bilirubin was elevated in two patients. Bromsulfalein retention was demonstrated in seven of fourteen patients. The prothrombin time was not significantly prolonged in any instance. Anemia was present in six subjects and was macrocytic in two.

It may be concluded that fatty infiltration of the liver occurs in a variety of conditions and presents variable clinical and laboratory findings, and requires needle biopsy for its diagnosis.

## 112. CHANGES IN TOLERANCE FOR GLUCOSE AND IN THE MORPHOLOGY OF PANCREATIC ISLET CELLS INDUCED BY INTRAVENOUS GLUCOSE IN DOGS

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(INTRODUCED BY HENRY R. JACOBS, M.D.)

The possibility that damage to the islets of Langerhans can be caused by the continuance over long periods of time of glucose infusions in nonhyperglycemic amounts was the object of the present study and report. Previous investigators have shown that there is a response on the part of the blood sugar regulating mechanisms to administered glucose. It was postulated that continuous administration even in nonhyperglycemic amounts would elicit such a response in such a way that these mechanisms would have no chance to "rest" and would become exhausted.

Glucose was administered to female dogs by continuous intravenous infusion, and the blood sugar changes followed. The pancreas was examined microscopically at the end of each experiment. Short-term infusions with hyperglycemic rates of glucose input have shown that such continuous infusions of glucose will cause an increase in the tolerance for glucose, as evidenced by the markedly lower blood sugar seen at the end of such an infusion as compared with the blood sugar seen shortly after the infusion is started. Long-term infusions (as long as forty-eight days) of amounts well below the hyperglycemic glucose input were shown to cause pancreatic islet cell damage with beta cell degranulation, congestion, and hydropic degeneration, and resulted in a much poorer glucose tolerance as evidenced by much higher blood sugar levels, in the hyperglycemic range, at the end of a long-term infusion, than were caused by the same amount of glucose in the early part of a given infusion.

The results of this investigation would seem to indicate that the tolerance for glucose can indeed be increased, and that such an increased tolerance stimulated by continuous intravenous glucose infusion will result in damage to the islet cells of the pancreas, and eventual loss of glucose tolerance, probably due to exhaustion of the insulin producing cells in the islets of Langerhans. This same microscopic picture has been reported before as the result of hyperglycemia maintained over long periods of time, by both anterior pituitary extract injections, and again by injected glucose. It would seem from the results of this investigation that the damage and loss of tolerance for glucose is due not to the hyperglycemia but to the continued stimulation of the blood sugar regulating mechanisms.

## 113. THE TREATMENT OF UREMIA BY DIALYSIS ACROSS THE INTESTINAL MUCOSA

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(INTRODUCED BY N. C. GILBERT, M.D., AND GEORGE C. TURNBULL, M.D.)

The effects of dialysis across the intestinal mucosa by lavage are studied in two cases of chronic terminal uremia. The first is one of congenital polycystic kidney, studied for two months. The other is a case of advanced nephrosclerosis in a woman whose right kidney had been removed surgically years ago, studied for one month. The irrigating solution is lactate-Ringer's with added dextrose 5 to 10 per cent. Levels of nitrogenous products in the dialysate from small bowel irrigation are equal to blood levels and the abstraction rate is inversely proportional to the rate of flow. A large inflow (priming) is required in intestinal dialysis before outflow effectively equals inflow. Irrigation of the lower ileum and colon is equally efficient and allows for control of edema. Alteration of irrigating solution concentration of glucose controls edema during irrigation. Glucose is absorbed during dialysis, necessitating concentrations up to 10 per cent in the irrigating fluid to maintain isotonicity. Sodium chloride in 0.6 per cent concentration is absorbed at all levels of the intestine, but sodium chloride deficient solutions will abstract as much as 0.5 Gm. per liter. In view of this, an irrigating solution containing 0.45 per cent sodium chloride would seem more suitable. Sodium lactate in the dialyzing fluid in combating acidosis, hemoconcentration, or dilation during dialysis did not influence blood nitrogen levels. By fastening a small-bored tube four feet from the tip of a Miller-Abbott tube, inflow of dialyz-

ing fluid occurs through the former, and outflow through the latter, which can be allowed to remain at the ileocecal level. This was shown to cause no irritation although in place for one month. Blood nonprotein nitrogen rises rapidly in the uremic individual following intravenous administration of protein hydrolysate.

## 114. A QUANTITATIVE STUDY OF THE SOLUBILITY OF HUMAN HEMOSIDERIN

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Previous studies of hemosiderin, isolated by drastic chemical methods, have indicated that the product is a form of ferric hydroxide. The present studies were an attempt to define the properties of hemosiderin by less drastic methods and to compare the properties of intracellular hemosiderin with those of chemically isolated hemosiderin and synthetic ferric hydroxide.

Hepatic tissue of patients with hemochromatosis and hemolytic hemosiderosis, secondary to multiple transfusions, was used. Frozen sections of fresh tissue were dried in vacuo for qualitative microscopic studies. Frozen pulverized fresh tissue was dried in vacuo for use in quantitative studies of solubility of hemosiderin. Sections for microscopic study and small amounts of powdered tissue were exposed to the action of buffer solutions of different ionic composition (pH 1.2 to 11; ionic strength, 0.25 and 0.50). Ferric hydroxide and hemosiderin isolated from hepatic tissue by the usual alkaline methods were exposed to similar solutions. Solubility was determined quantitatively by analyses of iron in supernatant solution and in the undissolved residue.

The data indicated that chemically isolated hemosiderin and ferric hydroxide had similar solubility properties. Hemosiderin in the cytoplasm of reticuloendothelial cells had solubility properties similar to but not identical with those of chemically isolated hemosiderin and ferric hydroxide. Hemosiderin in hepatic parenchymal cells had solubility properties which were very different from those of chemically isolated hemosiderin and ferric hydroxide. Intracellular hemosiderin, chemically isolated hemosiderin, and ferric hydroxide were almost insoluble in isotonic saline and phosphate buffer solutions at neutrality. A large fraction (50 to 80 per cent) of intracellular hemosiderin, especially that present in the cytoplasm of parenchymal cells, was soluble in citrate buffer solutions, ionic strength 0.50, at or near neutrality, while chemically isolated hemosiderin and ferric hydroxide were almost insoluble under the same conditions. Furthermore, within a part of the range in which intracellular hemosiderin had a high solubility (pH 6.1 to 7.0), crystals of unknown composition appeared in the tissues near the sites of granules of hemosiderin. These crystals did not appear in normal liver cells and were not formed in the presence of chemically isolated hemosiderin or ferric hydroxide. The results indicate that the problem of isolating one or more forms of hemosiderin by chemical extraction at neutrality, or after crystallization in situ, can now be approached.

## 115. INFARCTION OF THE MUSCLE BUNDLES OF THE HEART

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The object of this investigation was to determine whether myocardial infarcts are confined to the muscle bundles of the heart or occur without regard for them. For this purpose the topography of seventy-two infarcts in fifty unselected hearts was plotted and compared with the known topography of the four chief muscle bundles of the cardiac ventricles (superficial sinospiral, superficial bulbospiral, deep sinospiral, and deep bulbospiral muscles). It was found that all the infarcts followed definite patterns which coincided with the patterns of either one or more of the four muscle bundles. Twenty-five of the infarcts (35 per cent) in twenty-one cases (42 per cent) involved either a single superficial or a single deep bundle. The superficial muscles were involved, either alone or with a deep muscle, in 74 per cent of infarcts. The deep muscle bundles were involved, either alone or with a superficial muscle, in 51 per cent. Depending upon the thickness of the ventricular wall involved, it was possible to distinguish three types of infarcts: full thickness, massive but not full thickness, and laminar infarcts. Rupture, aneurysm formation, and mural thrombosis of the left ventricle were largely dependent upon the number of muscle bundles involved and the thickness of the infarct. Atrial infarcts were present in 42 per cent of the hearts. The immediate mortality of myocardial infarction was not influenced by the thickness of the infarct, the number of muscles included in it, nor by the involvement of any specific muscle.

## 116. INTERRELATION OF PTEROYLGLUTAMIC ACID AND VITAMIN $B_{12}$ IN INDUCED ANEMIA OF SWINE

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Swine maintained on a pteroylglutamic acid (PGA)-deficient diet, with "vitamin-free" casein as the source of protein, develop severe macrocytic anemia with megaloblasts in the bone marrow. Such animals respond well to PGA initially, but as the deficiency is maintained, the ability to respond to PGA diminishes. Administration of purified liver extracts then evokes a response. If liver extract or a source of "extrinsic factor" is given before PGA, a poor response can be elicited once or twice, after which no further response can be obtained until PGA is administered.

Two animals showed a slight response to relatively large doses of xanthopterin, but much less than to PGA.

One pig, maintained on the PGA-deficient regimen, for several months was given large amounts of liver extract and later vitamin $B_{12}$. There was an initial partial response, after which blood values were maintained better than previously but never became normal. Very marked macrocytosis developed and persisted. Administration of PGA then caused prompt disappearance of macrocytosis, and blood values became normal.

Vitamin $B_{12}$ deficiency was induced in swine by a diet containing alpha protein of soy beans, supplemented with methionine, as the source of protein; PGA was administered daily. Severe anemia developed in which macrocytosis was less marked and developed more slowly than in the PGA-deficient animals. The marrow did not contain megaloblasts. These animals responded to crystal-

line vitamin $B_{12}$. In one pig, there were peculiar double and triple reticulocyte peaks, each of which occurred after a single injection of vitamin $B_{12}$.

These findings indicate that PGA-deficient swine fed "vitamin-free" casein develop a deficiency of both PGA and the antianemia factor(s) of liver (solely vitamin $B_{12}$?). Either PGA or liver extract will induce a hematopoietic response, but both eventually are ineffectual unless the other is present. This indicates that both factors are required for normal hematopoiesis in swine.

While vitamin $B_{12}$ deficiency results in anemia, a deficiency of PGA appears to be importantly involved in macrocytosis and megaloblastosis. It is suggested that in human pernicious anemia a double deficiency also exists. The PGA deficiency may be the result of dietary inadequacy or of disordered metabolism of PGA. The deficiency of vitamin $B_{12}$ usually is dependent on an inadequacy of "intrinsic factor" in the gastric secretion but presumably can result from dietary deficiencies of vitamin $B_{12}$ and other factors.

## 117. CORRELATION BETWEEN RIBOSE NUCLEIC ACID DEPLETION AND OTHER SIGNS OF LIVER DAMAGE AS INFLUENCED BY VITAMIN $B_{12}$

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Disappearance of cytoplasmic ribonucleic acid compounds which are supposed to play a significant role in protein formation is one of the first signs of liver damage. Therefore, in comparison with twelve controls, the ribonucleic acid depletion (as recognized histologically) in thirty-six rats, forty-eight hours after intraperitoneal injection of a sublethal dose of carbon tetrachloride, was correlated with other signs of liver damage such as hydropic swelling of the parenchymal liver cells, increase of bromsulfalein retention, decrease of total liver proteins, and increase of histologically and chemically demonstrable fat. Chemical fractionation of total fat revealed the phospholipids remarkably constant, while all changes in the total fat were caused by variations of the neutral fat. Recent investigations had suggested that vitamin $B_{12}$ is important in the formation of ribonucleic acid compounds and subsequently it was demonstrated in this laboratory that the pathologic changes resulting from carbon tetrachloride ($CCl_4$) intoxication was significantly less pronounced in rats which had previously received very large doses of vitamin $B_{12}$. Therefore, forty-eight such protected rats were also included in the comparison.

In the individual animals of the intoxicated as well as the protected group, there was a parallelism in the degree of the different alterations. This parallelism was marked between the degree of fatty deposition and bromsulfalein retention. The increased bromsulfalein retention was found more related to circulatory embarrassment by the fat deposition (as judged from the presence of erythrocytes in the sinusoids) than to liver cell damage. On the other hand, there was an almost as marked parallelism between liver cell damage, ribonucleic acid depletion, and decrease in liver protein.

Since with small doses of $CCl_4$ ribonucleic acid depletion in the center of the lobules can be produced without other evidence of liver damage, the ribonucleic acid depletion is apparently one of the basic factors in this type of liver damage. Moreover, vitamin $B_{12}$ seems to inhibit liver damage by facilitating the formation of ribonucleic acid. That is probably related to the assumed role as synthetizing ribonucleases of growth factors such as vitamin $B_{12}$. Their influence on protein formation in turn may control the enzyme regeneration in liver damage.

These experiences suggest the investigation of the effect of vitamin $B_{12}$ in different types of liver diseases. In view of the relatively large doses necessary for effect in rats, variations in vitamin $B_{12}$ content possibly explain the erratic results obtained with liver extracts in these disorders.

## 118. RONIACOL—A VASODILATOR SUBSTANCE CONVERTED IN THE ORGANISM TO NICOTINIC ACID

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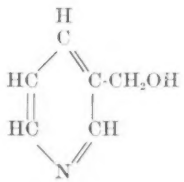
Roniacol, a vasodilator substance which is converted in the organism to nicotinic acid, is 3 pydridine-methanol or B-pyridyl-carbinol (the alcohol corresponding to nicotinic acid) and has the following structural formula:




During the experimental period, the code designation for the compound was Nu 2121.

Roniacol is a nonvolatile solid freely soluble in water and in alcohol and of low toxicity for animals. Administration of the human dosage to dogs for three months produced no adverse effect on weight, blood formation, or nonprotein nitrogen. In all animals, powerful vasodilator effect on both coronary and peripheral circulation was observed.

There are several conditions in which prolonged vasodilation should be of benefit particularly in treatment but also in diagnosis. These include conditions in which coronary, renal, or peripheral cerebral vascular spasms are involved. Collateral circulation is of paramount importance when the vascular lumen is narrowed.

To be effective in aiding nutrition in an area with deficient vascular supply, brief and evanescent dilation in collateral vessels requires frequent repetition of the dilator effect. A prolonged dilator effect is to be sought and should aid in establishing permanent dilatation of the vessels involved.

Relatively free from uncomfortable or deleterious effects, and capable of causing an immediate, visible flushing of the skin when given by mouth, the drug can be repeated as often as desired.

This preliminary report is concerned with two conditions in which vasospasm and vasodilation in a collateral circulation are important, the angina pectoris syndrome and peripheral vascular lesions.

In angina pectoris, Roniacol has appeared repeatedly to increase tolerance for exercise and to extend the range of activity without occurrence of pain. In peripheral vascular disease the drug has been used to determine whether the total circulation of the involved parts can be improved; and, in cases showing improvement, sympathectomy may be expected to be effective.

Cases illustrative of the foregoing conditions are cited.

## 119. RAPID TREATMENT OF ACUTE GOUTY ARTHRITIS BY CONCURRENT ADMINISTRATION OF PITUITARY ADRENOCORTICOTROPIC HORMONE (ACTH) AND COLCHICINE

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(INTRODUCED BY SAMUEL SOSKIN, M.D.)

Clinical gout appears to require two endocrine disturbances. An abnormal male sex hormone seems to regulate appearance of inherited hyperuricemia. Gout patients also appear relatively unable to meet acute relative glycocorticoid lack with a prompt increase in production. When glycocorticoid lack occurs, it tends to persist and to precipitate acute gouty arthritis. Hellman and Robinson, Conn, Block, and Louis induced acute glycocorticoid lack by administering ACTH in interval gout and then withdrawing this hormone and found the procedure to precipitate acute gouty arthritis in a majority of patients. Their observations have been confirmed.

ACTH, given during acute gouty arthritis, repairs the glycocorticoid lack and rapidly terminates the episode. However, given alone, ACTH is not satisfactory therapy for acute gouty arthritis. In most patients the relative glycocorticoid deficiency which follows hormone withdrawal precipitates a renewed attack shortly after withdrawal.

Colchicine, given during or immediately after treatment of acute gouty arthritis with ACTH, effectively overcomes the tendency of ACTH withdrawal to precipitate a renewal of the attack. Single 50 mg. doses of ACTH (Armour) have effectively terminated most attacks within four hours. Occasionally a second or third dose has been required at six-hour intervals after the first. Although a number of the episodes treated had been prolonged and resistant to previous therapy, no ACTH-treated attack has persisted more than twenty-four hours after hormone therapy was begun.

Colchicine administration has been begun either with ACTH administration or four hours following the last dose of ACTH. All patients have been colchicinized to tolerance and maintained on daily subtolerance doses of colchicine for at least two weeks. No patient who has received combined ACTH-colchicine therapy has had even a minor recurrence of acute gouty arthritis within one month of treatment.

Concurrent administration of ACTH and colchicine appears to be the most rapid and effective available treatment for acute gouty arthritis. In view of the relative scarcity of ACTH, the small amounts of this hormone which are required constitute a particularly desirable feature.

## 120. EFFECT OF HYPERVENTILATION ON THE HEMORESPIRATORY EXCHANGE IN NORMAL PERSONS, PATIENTS WITH PULMONARY EMPHYSEMA, AND PATIENTS WITH CARDIAC DYSPNEA

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MINNEAPOLIS, MINN.

Respiration aids in maintaining a normal blood pH by regulating the tension of carbon dioxide in the alveoli and hence in the arterial blood. Hyperventilation in normal persons produces alkalosis by sudden lowering of the alveolar and blood $pCO_2$.

The purpose of this study was to determine the response to voluntary hyperventilation of patients with dyspnea secondary to heart disease or chronic pulmonary emphysema. In twelve normal persons, fourteen cardiac patients, and twelve patients with pulmonary emphysema the total lung volume and its subdivisions, the minute volume, oxygen uptake, $CO_2$ elimination, arterial blood pH, $CO_2$ content, and oxygen saturation were measured in the basal state and after two minutes of voluntary hyperventilation. The $pCO_2$ of arterial blood was estimated from the nomogram of Singer and Hastings.

In the group of patients with emphysema the residual air was markedly increased with a corresponding reduction in vital capacity, the total lung volume being normal. Patients with heart disease showed a reduction in vital capacity and total lung volume with a normal residual air.

Mean values before and after hyperventilation in normal persons and patients with cardiac dyspnea were very similar. In the group of patients with emphysema the initial $pCO_2$ was elevated and the oxygen saturation was diminished, the value for the former being 50 mm. Hg and for the latter 83 per cent. The blood pH was normal as the result of a compensatory increase in plasma bicarbonate. Voluntary hyperventilation was relatively ineffective in this group and did not significantly alter the initial values.

Hence, patients with cardiac dyspnea like normal persons have ventilatory control over blood pH. Inability of patients with emphysema to increase alveolar ventilation effectively by maximal effort results in serious impairment of ventilatory regulation of blood pH. This defect renders these patients particularly vulnerable to metabolic and respiratory acidosis and is a constant threat to life.

## 121. A SINGLE SCALE ABSOLUTE READING EAR OXIMETER

EARL H. WOOD, M.D., ROCHESTER, MINN.

(INTRODUCED BY C. F. CODE, M.D.)

Two modified oximeter earpieces were calibrated on the basis of their responses on empirical optical filters using the single scale circuit described by Wood and Geraci.

Earpiece 1 was used to determine arterial oxygen saturation on thirty-three ears of eighteen normal subjects. Thirteen were white and five were Negroes. Ages ranged from 2 to 50 years. The average saturation obtained when the subjects were breathing air was 96.7 ± 0.3* (93-101) per cent and 99.4 ± 0.4 (94-106) per cent when breathing oxygen.

Earpiece 2 was used on twenty-four ears of seventeen normal subjects. Twelve were white, two were Chinese, and three were Negroes. The average arterial saturation was 96.7 ± 0.3 (94-100) and 99.7 ± 0.5 (96-104) per cent when the subjects were breathing air and oxygen, respectively.

Eighteen simultaneous Van Slyke and oximetric determinations of arterial oxygen saturation were made on six patients with congenital heart disease during supine rest, breathing air or oxygen, standing and walking. The per cent arterial oxygen saturation by Van Slyke analysis of radial artery blood averaged 85 and ranged from 39 to 100 per cent. The standard deviation of the differences between simultaneous Van Slyke and ear oximeter determinations in these hypoxemic patients was 4 per cent.

*The number following the ± sign is the standard error of the mean, N = 33.

The instrument can be A.C. or battery operated, is compact, relatively inexpensive, simple to operate, and is apparently accurate enough to warrant its use for most clinical estimations of arterial oxygen saturation.

## 122. STUDIES OF HEMAGGLUTININS IN CONGENITAL AND ACQUIRED HEMOLYTIC ICTERUS

CLAUDE-STARR WRIGHT, M.D., MATTHEW C. DODD, PH.D. (BY INVITATION), AND BERTHA A. BOURONCLE, M.D. (BY INVITATION), COLUMBUS, OHIO

WITH THE TECHNICAL ASSISTANCE OF CHARLES A. CUNNINGHAM, B.S.

The immunohematologists studying the Rh mechanisms developed a series of techniques for demonstrating incomplete (blocking) antibodies which have proved useful in investigating other hemolytic anemias. To the Coomb's, "developing" and "conglutination" tests, Wheeler, Luhby, and Scholl have recently added a technique,* previously suggested by Burnet, Pickels, and Quilligan, using enzyme-treated normal red blood cells for detecting incomplete Rh antibodies in sera. They found the technique a simple, reliable, sensitive, inexpensive, and rapid screening test for demonstrating incomplete Rh antibodies in sera of Rh-negative women. We have added this to the battery of techniques in the study of congenital and acquired hemolytic icterus. The following observations have been made: (1) 255 normal sera gave no agglutination with trypsinized red blood cells. (2) In twenty cases of acquired hemolytic icterus, one or both tests were positive in sixteen cases; fourteen with trypsinized red blood cells; fourteen with Coomb's serum. (3) In twenty-one cases of congenital hemolytic icterus, one or both tests were positive in eight cases; six with trypsinized red blood cells; four with Coomb's serum.

It is concluded that (1) the trypsinized red blood cell test is a valuable adjunct for demonstrating incomplete antibodies in acquired and congenital hemolytic icterus. (2) In acquired hemolytic icterus the avidity and titers of the trypsinized red blood cell test are generally greater than with the Coomb's test; however, they run a closer parallel in acquired than congenital types. (3) The Coomb's test, contrary to recent claims, has not been a reliable means of differentiating acquired and congenital hemolytic icterus thus far in our hands.

## 123. CAVITY POTENTIALS OF THE HUMAN VENTRICLES

HENRY A. ZIMMERMAN, M.D., AND HERMAN K. HELLERSTEIN, M.D., CLEVELAND, OHIO

(INTRODUCED BY ROY W. SCOTT, M.D.)

The intracavitary potentials of the human left ventricle have been studied by retrograde arterial catheterization. In eight men, 48 to 74 years of age, the left ventricle was entered successfully in six, while in two the tip of the

*The trypsinized red blood cell technique (modified after Wheeler): 1 ml. of washed (three times in cold normal saline) packed type "O" red blood cells is added to 1.5 ml. of a 0.1 per cent trypsin (Difco 1:250 activity trypsin) saline (buffered to pH 7.2) solution. Suspension incubated thirty minutes, 37° C., with frequent agitation. Centrifuge, remove supernatant, and wash enzymized red blood cells three times with cold saline. Make a 2 per cent cell suspension in saline. Suspension is checked for nonspecific agglutination with normal sera.

Serial dilutions of the patient's serum are made with 0.1 ml. of serum and 0.1 ml. of saline. Two drops of the above suspension of trypsinized red blood cells are added. Incubate at 37° C., thirty minutes, and read for agglutination.

Normal sera are run as controls.

catheter electrode was obstructed by the aortic valve. All patients had aortic insufficiency, with an average pulse pressure of 98 mm. Hg. The right ventricle potentials were obtained by venous catheterization.

In the region of the upper part of the interventricular septum, curves from the right ventricle showed a small R wave and a deep S wave; in the corresponding region of the left ventricle, the complex was of the QS variety, with an initial slurring on the downward limb corresponding to the R wave of the right ventricular lead. In no case was a positive deflection recorded in the left ventricle in this region. These findings are consistent with the current belief, proposed by Wilson, that the upper part of the interventricular septum is depolarized from left to right.

In one case, however, curves from the apex of the left ventricle showed a definite R wave and a deep S wave. The origin of this R wave is obscure and may be due to (1) depolarization of the lower septum from right to left, or (2) to the tip of the catheter electrode being situated in the apex which would be "facing" the wave of depolarization passing down the Purkinje system of the left ventricle.

Premature beats were most frequently produced when the catheter first entered the cavity of the left ventricle in the upper septal region. In several cases runs of ventricular beats occurred for two to four seconds, spontaneously disappearing when the catheter was withdrawn or passed forward toward the apex. Premature beats originating from the endocardium of the left ventricle produced a wide QS complex, and usually a positive QRS complex in the right and left arm unipolar leads and a negative QRS complex in the left foot unipolar lead.

Tracings were also obtained above the aortic ring and in the arch of the aorta. The ventricular complexes resembled those of the right arm unipolar lead. Thus in a patient with ST elevation in aVr, and depression of the ST segment in $V_{3\text{-}6}$, and in $aV_1$, the ST elevation in the lead from the arch of the aorta indicated that essentially cavity potentials were being recorded in aVr and aortic leads. However, aVr did not resemble the intracavitary lead in all cases. One patient with marked left axis deviation and left ventricular hypertrophy had positive aVr and $aV_1$, but the cavity leads of the right and left ventricles had net negative values.

## 124. A STUDY OF PULMONARY HEMODYNAMICS DURING PNEUMONECTOMY

### A Preliminary Report

Henry A. Zimmerman, M.D., Harvey Mendelsohn, M.D., and Arthur Adelman, M.D., Cleveland, Ohio

(Introduced by Roy W. Scott, M.D.)

Cournand has studied pulmonary hemodynamics before and after pneumonectomy in human beings, but there are no published data on the changes occurring during the actual operative procedure. We have studied five patients undergoing pneumonectomy by the technique of right heart catheterization during the operation, when a sudden burden may be expected to be thrown on the vascular bed of the remaining lung following ligation of the opposite vessel. A catheter is placed in the pulmonary artery opposite to the one to be ligated sixty minutes before the induction of anesthesia. Basal cardiac output and initial pressures are recorded and subsequent pressure

levels are determined at fifteen-minute intervals throughout the entire procedure, including the period immediately following ligation of the pulmonary artery. Intra-arterial brachial pressure, phase of respiration, pulmonary arterial pressure, and the electrocardiogram are recorded simultaneously on a six-channel oscillograph described elsewhere. In two patients who had lesions which were not resectable, pulmonary artery pressure measurements were obtained after temporary ligation of one pulmonary artery.

We have demonstrated a consistent average rise in pulmonary arterial pressure of 50 per cent above the basal level in all instances immediately following ligation. Pulmonary artery pressures dropped to near basal levels by the end of the operation in the three patients in whom resections were done. This took an average time of forty-five minutes. These findings agree with the recent work of Long and associates in dogs.

We believe that the preoperative measurement of pulmonary arterial pressure is an important adjunct in the evaluation of the patient's suitability for pneumonectomy. This immediate rise in pulmonary arterial pressure, with the sudden load thrown on the right ventricle, may be responsible for unexpected deaths which occur after ligation of a pulmonary artery.

Scott and Zimmerman have shown that elevated pulmonary arterial pressures due to a variety of causes may be lowered by aminophylline. The effect of this drug administered prior to ligation of the pulmonary artery is under investigation.

# The Journal of Laboratory and Clinical Medicine

**The Official Publication of the Central Society for Clinical Research**

CLAYTON G. LOOSLI, M.D., Editor
University of Chicago School of Medicine
950 East 59th Street Chicago 37, Illinois

PUBLISHED BY THE C. V. MOSBY COMPANY, 3207 WASHINGTON BLVD.,
ST. LOUIS 3, U. S. A.

Published monthly. Subscriptions may begin at any time.

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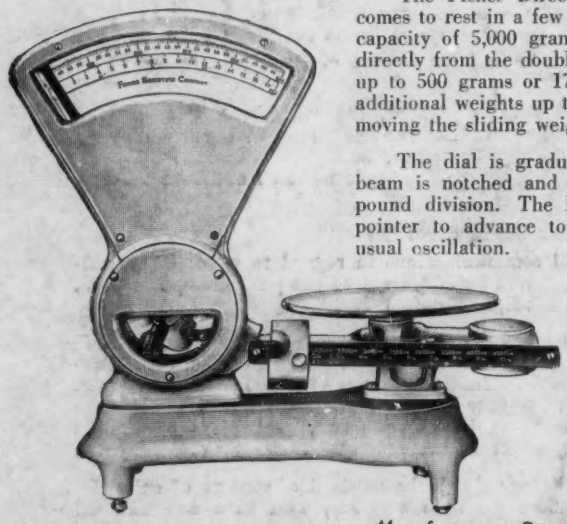

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